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ERRATA AND AUTHORS' EMENDATIONS

Page 17, line 7, "at chlorosis" should be "a chlorosis."
Page 37, table 2, column 3, heading, "4-lock bolls" should be "5-lock bolls."
Page 41, twenty-third line from bottom, omit the words "squares and" between "of" and "young."
Page 107, after "Materials and Methods" add footnote reference 3; at bottom of page insert the following as footnote 3: "Nitrogen equivalent indicated refers to grams per liter of NO_3 or NH_4 radical."
Page 132, table 3, footnote *b* should read "Hope×Ceres, N.N. 1127."
Page 227, line 20, the words "and is soon completed" should appear between "(fig. 7, B)" and "except" (line 19).
Page 233, line 11, "1928" should be "1828."
Page 387, table 11, column 3, line 1, in place of leaders insert "72.6", and in line following omit "31.5."
Page 390, table 12, column 2, line 1, change "31.5" to "72.6."
Page 405, in formula (1) $\frac{S(fd)^2}{n^2}$ should be $\frac{(Sfd)^2}{n^2}$.
Page 647, legend for figure 2, "(See table 7.)" should be "(See table 5.)"
Page 732, in the first of the two equations in the final paragraph, the numerator of the right-hand member "σ^2 12.3" should be "σ^2 1.23."
Page 742, line 22, "In one pen" should be "In four pens."
Following page 760, plate 3, legend, line 4, "*B*" should be "*A*."
Page 770, Literature citation (15), "V.1" should be "V.3."
Page 775, line 19, "5" should be "3."
Page 788, line 3, "central Mexico" should be "Central America."
Page 861, 5th line from bottom, omit "and *A*" after "*B*."
Page 913, table 1, column 1, line 5, "300 15:15:15:15" should be "300 15:15:15:."
Page 957, line 13, "raised on" should be "fed."
Page 962, line 2, "milk-cured" should be "milk-curd."
Page 999, table 1, column 2, "Sporophores" should be "Sporophore"

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FURTHER CYTOLOGICAL STUDIES OF HETEROTHALLISM IN PUCCINIA GRAMINIS[1]

By RUTH F. ALLEN[2]

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INTRODUCTION

An earlier paper (*1*)[3] presented the results of a cytological study of the aecial generation of *Puccinia graminis* Pers. on the barberry (*Berberis vulgaris* L.). Stages of the entrance of the fungus and its development within the host plant were traced and an attempt was made to show its mode of sexual reproduction. *P. graminis* is heterothallic. Infections that are kept isolated remain gametophytic (with a few doubtful exceptions) and produce no aeciospores. The spermatia (pycniospores) function in effecting fertilization. The exact mode of entrance of the spermatia was not determined, but the frequent occurrence of cells with two or more nuclei in the walls of the spermogonia (pycnia) and in adjoining mycelium suggested the possibility that the paraphyses might serve to receive the spermatia. The sporophyte generation, once initiated, spreads from its point or points of origin through the mycelium. Aecia borne by such mycelium commonly contain sporophytic cells from their inception.

This paper presents the results of a more intensive study of the surface hyphae and related details in *Puccinia graminis*. For this study, slides were used that had been prepared during the earlier study and additional slides were made from material then embedded in paraffin.

INVESTIGATIONS

The rate of growth of the young infection varies greatly with environmental conditions and with the age of the host leaf. Four days after inoculation, under the most favorable conditions, the fungus has entered, has formed the primary hypha in a cell of the upper epidermis, has spread from this through the intercellular spaces of the mesophyll to the lower surface of the leaf, and has begun reproductive activities.

Plate 1, A, shows semidiagrammatically a cross section of a young barberry leaf, with thin-walled cells and small air spaces, that bears a typical well-grown 4-day infection. The leaf is drawn in outline only, and the fungus is shaded to bring it out more clearly. The hyphae have permeated the intercellular spaces of the mesophyll. At two

[1] Received for publication Dec. 9, 1932; issued August 1933. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and the Agricultural Experiment Station, University of California.

[2] Acknowledgments are made to H. B. Humphrey, of the Division of Cereal Crops and Diseases, for careful reading of this manuscript and for barberry plants and spores; to W. P. Fraser, of the University of Saskatchewan, Canada, for spores; and to members of the Divisions of Agronomy and Genetics of the University of California for courtesies extended during the work.

[3] Reference is made by number (italic) to Literature Cited, p. 14.

points at the upper surface (*a*, *b*) and one at the lower (*d*) young spermogonia are forming. At *c*, a hypha has grown up between cells of the upper epidermis and flattened itself against the cuticle. There are no hyphae in the stomata *d*, *e*, *f*, which appear to be closed, but at *e* a hypha is pushing down between a guard cell and the next epidermal cell.

A detailed study has been made of the hyphae that grow to the upper and lower surfaces of the leaf. Plate 1, B to G, shows typical surface hyphae found in 4-day infections. Plate 1, B, represents a detail at the upper surface of the leaf. Between the epidermis (*b*) and the palisade (*d*) there is a growth of mycelium (*c*, *e*) which is continuous at the right with a young spermogonium (beyond the limits of the drawing). From this subepidermal mycelium a hypha (*a*) has forced a passage up between the epidermal cells and has grown a short distance along the outer face of the epidermal cell beneath the cuticle. Plate 1, C, shows a similar surface hypha with a more extended subcuticular growth at *a*. Plate 1, D, *a*, shows a cross section of such a surface hypha at the lower epidermis. In all three (B, C, D) it is evident that the growing tip of the hypha has been able first to split the common wall between two epidermal cells at the middle lamella and grow out between the two to the surface and then separate the cuticle from the rest of the outer wall and force a passage there. Whether this is achieved by chemical or mechanical processes is not clear, but the latter seems probable.

At this time (four days after inoculation) hyphae are to be found about the stomata in the lower epidermis of the leaf, but rarely is one thrust between the guard cells. Plate 1, E, shows a bit of the lower epidermis, including a longitudinal section of a stoma (*b*). A hypha (*a*) is wedged firmly into the angle between the guard cell and the next epidermal cell. Another hypha (*c*), after growing down into the angle between the guard cells but failing to enter the stoma, has grown on to *d*. Perhaps the stoma is closed. Plate 1, F, shows a cross section of a stoma. One hypha (*a*) is pressed into the angle between the guard cells of the closed stoma, another (*b*) is forcing a passage between the guard cell and the next epidermal cell. The walls adjoining stomata seem to be a particularly vulnerable part of the young leaf's defenses. Plate 1, G, shows another stoma with a hypha growing down toward the lower surface at each end of the guard cell (*a*, *c*). At *b* is one of the very few examples of a hypha that has entered the stomatal opening.

These hyphae that emerge at the surface of the leaf resemble the receptive hyphae seen in other rusts (*2*, *3*, *4*, *5*). At this stage, however, the infection, if visible macroscopically, is only a minute spot a

EXPLANATORY LEGEND FOR PLATE 1

A.—Semidiagrammatic drawing of central section through 4-day infection of a barberry leaf by *Puccinia graminis;* fungus shaded. Young spermogonia at *a*, *b*, and *d;* surface hypha at *c;* hypha growing out between an epidermal cell and a guard cell of the adjoining stoma at *e;* hyphae near stoma at *f*. × 320.

B.—Between the upper epidermis (*b*) and the palisade (*d*) are hyphae (*c*, *e*) from which a hypha (*a*) has grown up to the leaf surface and a short distance under the cuticle. Four-day infection. × 1,020.

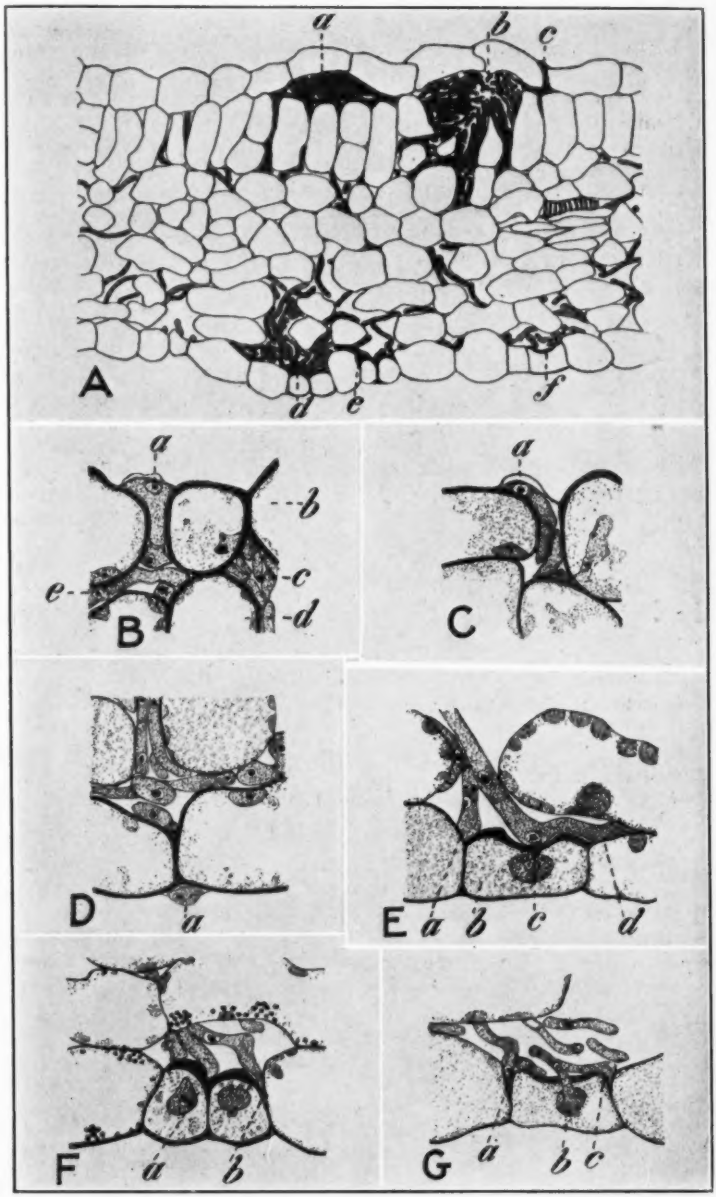
C.—Similar to B; longer subcuticular growth at *a*. × 1,020.

D.—Detail of lower epidermis of 4-day infection. Cross section of subcuticular hypha at *a*. × 1,020.

E.—Detail from 4-day infection, showing longitudinal section of stoma (*b*). A hypha (*a*) is wedged into the angle at the end of the guard cell; another hypha has pressed into the hollow of the stoma at *c* and has then grown on to *d*. × 1,020.

F.—Detail of lower epidermis of 4-day infection. A hypha (*a*) is wedged into the angle between the guard cells; another hypha (*b*) is forcing a passage outward at the side of the stoma. × 1,020.

G.—Longitudinal section of stoma. Hyphae (*a*, *c*) are growing down at ends of guard cell; another hypha (*b*) is inserted in stomatal aperture. × 1,020.



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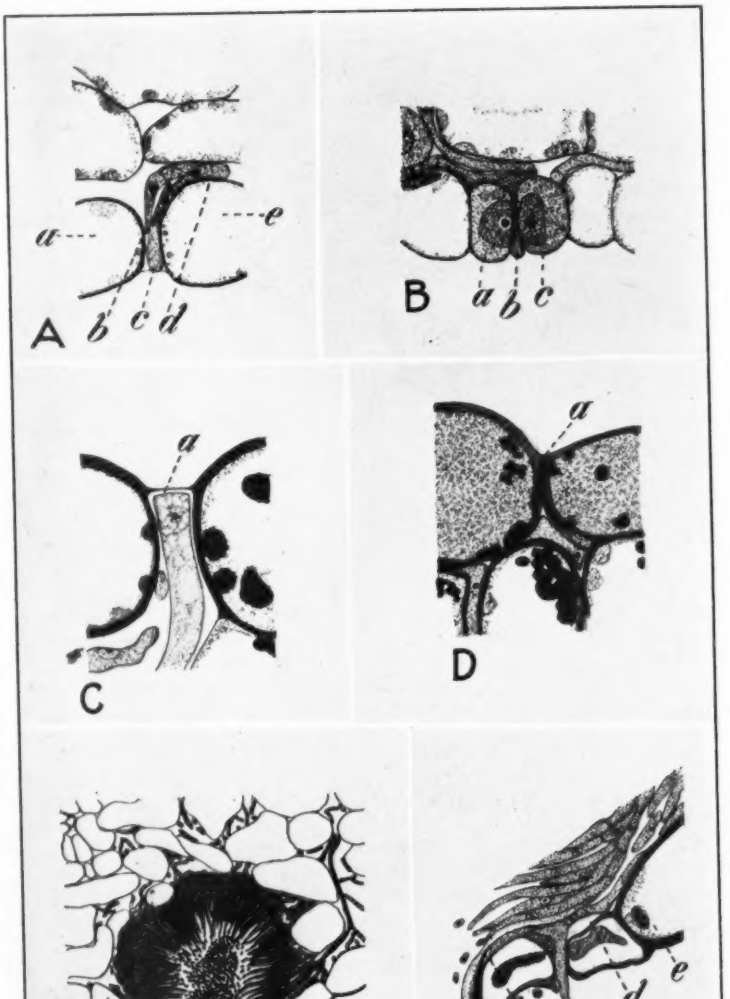

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shade lighter in color than the rest of the leaf. There are no mature spermogonia; consequently, there is no spermogonial exudate. There is nothing to attract the insects that might bring nectar from other infections and effect fertilization. These surface hyphae appear to be functional receptive hyphae, but there is small chance that fertilization would occur at this stage under natural conditions, and no case has been noted in the greenhouse material.

During the fifth and sixth days after inoculation the mycelium spreads farther in the mesophyll, the first spermogonia mature and open, and in a few of the most rapidly growing infections a rare aecial primordium forms.

If the infection is located in a young leaf with soft thin walls, the hyphae growing out to the surface remain unchanged. Plate 2, A, from a 5-day infection, shows a detail of the lower epidermis. Between the two epidermal cells *a* and *e*, a hypha (*c*) has grown down to the cuticle and other hyphae (*b*, *d*) are wedging in beside it. Plate 2, B, shows another bit of the lower epidermis. Between the still thin-walled guard cells (*a*, *c*) of the stoma, a hypha (*b*) has grown down flush with the outer surface of the leaf. This hypha is markedly constricted by the closing of the stoma.

When the young infection is growing in an older leaf, the thick, tough epidermal walls offer more resistance to the outward growth of hyphae. Under the conditions shown in plate 2, C and D, the formation of emergent hyphae obviously would be more difficult than in the examples already described.

Plate 2, C, *a*, shows a hypha that has grown up between two cells of the upper epidermis. The large size of the hypha and the conformation of the epidermal wall about its apex suggest that the hypha may have formed when the host walls were thin (compare with pl. 2, A) and that as the epidermal wall thickened it shaped itself about the hypha. In plate 2, D, on the contrary, a slender hypha (*a*) has worked its way out between the layers of the heavy epidermal walls almost to the surface and has then died.

Plate 2, E and F, shows another type of receptive hypha formed in connection with a spermogonium. Plate 2, E, represents semidiagrammatically a newly matured spermogonium. The interior portion consists of a nearly spherical shell or wall (*a*) from which numerous spermatiophores (*b*) grow into the central cavity, and paraphyses (*c*) grow to the ostiole and out to form the extruded brush (*e*, *f*). Spermatia are set free into the central cavity (*d*) and ooze out through the ostiole to form the external exudate. The growing spermogonium lifts and stretches the epidermis adjoining it and puts it under strain.

EXPLANATORY LEGEND FOR PLATE 2

A.—Detail of 5-day infection on a young barberry leaf. Between two adjoining cells (*a*, *e*) of the lower epidermis a hypha (*c*) has grown down to the cuticle; other hyphae (*b*, *d*) are wedging in beside it. × 1,020.

B.—Detail of 6-day infection on young leaf. Between the guard cells of the stoma (*a*, *c*) a hypha (*b*) has grown down to the lower surface. × 1,020.

C.—Detail of 6-day infection on older leaf. A broad surface hypha (*a*) has grown between two cells of the upper epidermis. × 1,020.

D.—Detail of 6-day infection on older leaf. A slender hypha (*a*) has forced a passage up between heavy-walled epidermal cells and has then died. × 1,020.

E.—Newly opened spermogonium on lower surface of 6-day infection. Wall of spermogonium (*a*) gives rise to spermatiophores (*b*) and paraphyses (*c*, *e*, *f*). Spermatia (*d*) are freed in central cavity and ooze out through ostiole. Surface hypha at *g*. Epidermal cell at *h*. × 320.

F.—Area from *f* to *h* of Plate 2, E, enlarged. Beneath the epidermis (*e*) are spermatiophores and the bases of paraphyses which continue out to form the extruded brush at *a*. From the young paraphysis (*d*, *b*) a branch (*c*) has pushed out between epidermal cells to the leaf surface. × 1,020.

Because of this weakened condition of the epidermis, hyphae frequently grow out between epidermal cells close to the ostiole (pl. 2, E, *g*).

The area *f* to *h*, in plate 2, E, is drawn enlarged as plate 2, F. At *a* is a portion of the extruded brush of paraphyses with the adherent exudate. At *d* a young paraphysis has forked, one branch (*b*) going on beneath the epidermis toward the ostiole, the other (*c*) growing out between epidermal cells to the surface. The hypha *c* may be regarded as either a receptive hypha or a displaced paraphysis.

The spermogonium just described opens upon the lower surface of the leaf. This is a common occurrence; a count of spermogonia in 7-day and 8-day material shows that 38 out of 117 (about one-third) open upon the lower side of the leaf.

In 7-day infections the surface hyphae are unchanged, except that there may be an increase in the subcuticular growth, particularly if the host leaf is young. Plate 3, A, shows a low-magnification diagram of a portion of a young barberry leaf. A detail at *c* is shown enlarged as plate 3, B. The hypha *a*, from the bases of the paraphyses, grew to the surface at *b* and along the surface for a considerable distance to *c*. It is not clear whether it is inclosed by the cuticle. Sometimes even more extended surface growth is found; and in at least one case it was two cells thick.

At this stage the stomata serve but rarely as outlets of surface hyphae. Plate 3, C, shows a stoma occupied by hyphae, but the shutting of the stoma has nearly pinched off the hyphae. More successful is the surface hypha at one end of the stoma. Plate 3, D, shows the hypha *a* between a guard cell (cut obliquely)and an adjacent epidermal cell.

To the statement that the stomata are of little importance, an exception should be made regarding stomata adjoining a spermogonium. Plate 3, E, drawn from an 8-day infection, shows a lateral section through a spermogonium, cut crosswise through the bases of paraphyses and spermatiophores. The stoma (*a*, *c*) is wide open, perhaps because of the tension on the epidermis produced by the spermogonium, and in the stomatal aperture (*b*) are no less than five vigorous hyphae.

It has been noted that the progress of development of an infection varies considerably according to the age of the leaf in which the infection is growing, for older barberry leaves are very firm in texture and the epidermis becomes very heavy. Parallel studies have been made of two unfertilized 9-day infections, one growing on a fairly young leaf and the other on an old leaf. Table 1 shows the number and location of the receptive hyphae in the two infections.

EXPLANATORY LEGEND FOR PLATE 3

A.—Diagram of section of a barberry leaf through a 7-day infection. Young spermogonia at *a*, *b*, and *c*. × 115.

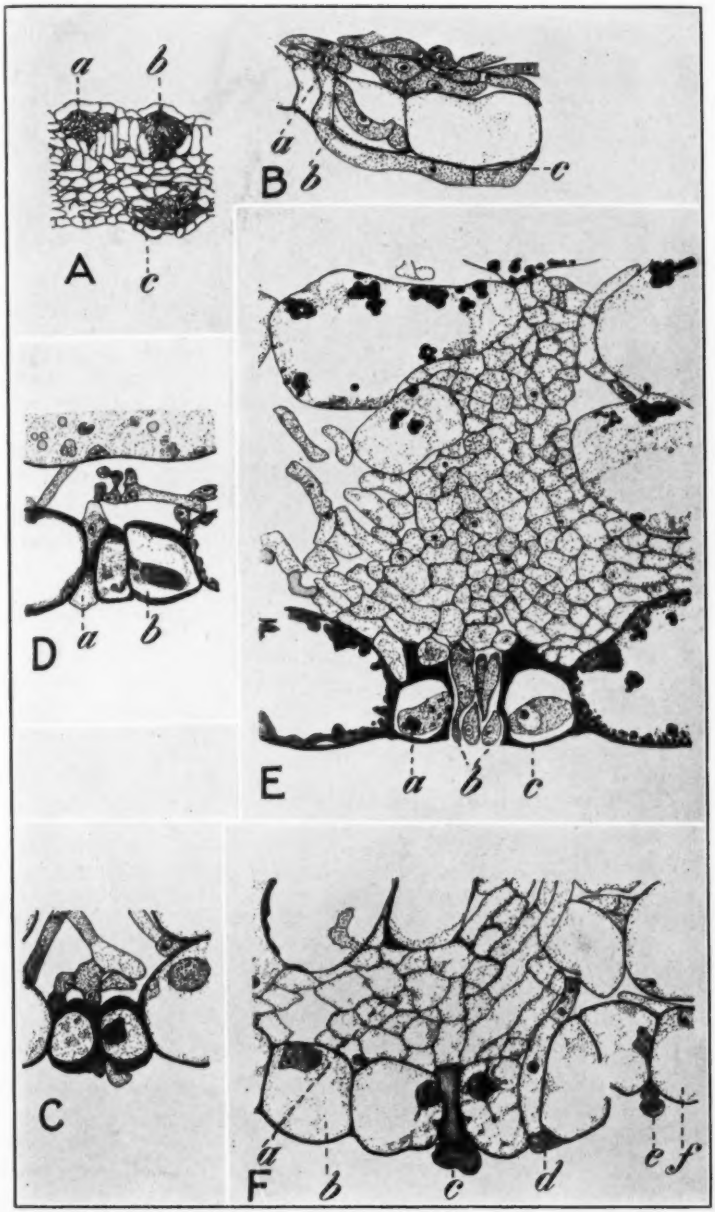
B.—Detail at A, *c*, enlarged, showing young paraphyses above the epidermis, from which a hypha (*a*) grows down to the outer surface at *b*, and along the surface to *c*. × 1,020.

C.—Bit of lower epidermis of 7-day infection, showing stomatal hyphae constricted by closure of stoma. × 1,020.

D.—Stoma (*b*), cut obliquely, from 7-day infection. A hypha (*a*) has grown down at one side of the stoma to the outer surface. × 1,020.

E.—Detail from 8-day infection. Stoma (*a*, *c*) forced wide open by pressure of growing spermogonium above it. Stomatal aperture (*b*) filled with hyphae. Inner walls of guard cells deformed. × 1,020.

F.—Detail from 9-day infection. Lateral section through spermogonium at *a*, above lower epidermis (*b*, *f*). Surface hyphae between epidermal cells at *c*, *d*, and *e*. × 1,020.



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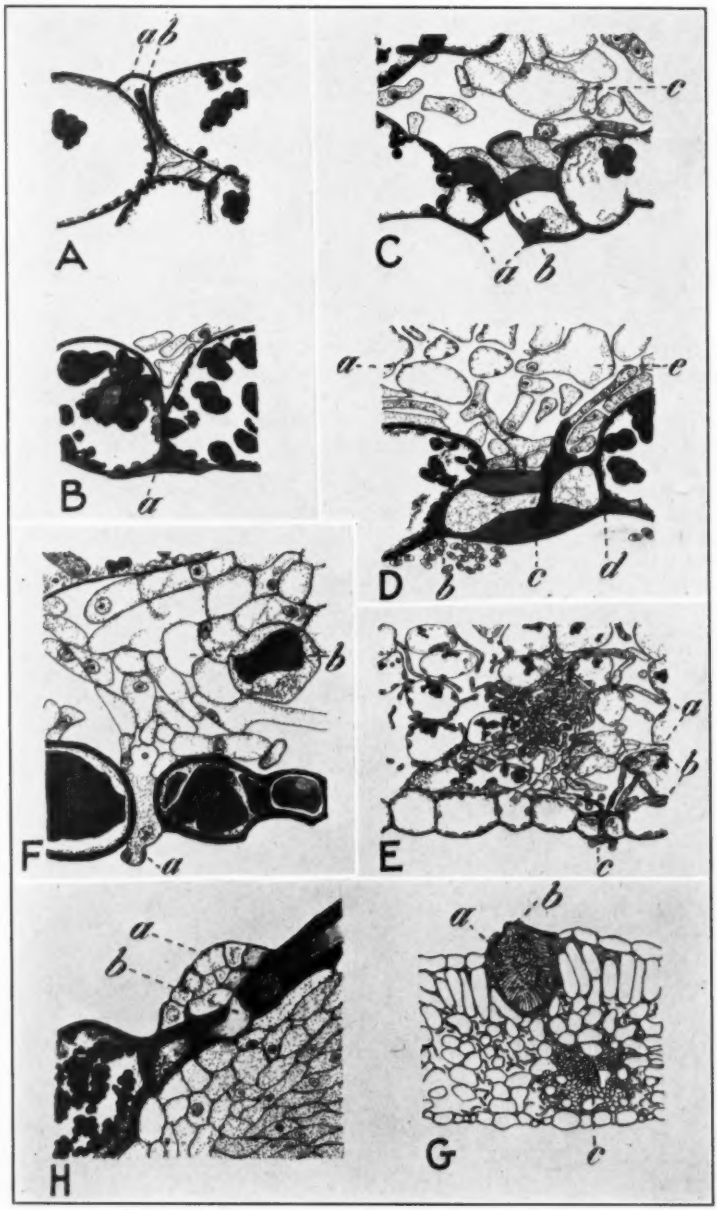

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TABLE 1.—*Number and location of receptive hyphae in two 9-day infections*

Infection on—	Upper surface		Lower surface			Total
	Adjoining a spermogonium	Remote from a spermogonium	In stomata	Between epidermal cells		
				Adjoining a spermogonium	Remote from a spermogonium	
Fairly young leaf	7	8	2	27	8	52
Old leaf	1	9	[a] 10	1	4	25

[a] Of these, 6 were located in stomata adjoining or near young aecia.

In the 9-day infection on the younger leaf, 37 out of 52 (about 70 percent) of the surface hyphae are at the lower surface of the leaf and 27 (about 50 percent) are between lower epidermal cells close to the ostiole of a spermogonium. Plate 3, F, shows a typical example. The lateral section through the spermogonium at *a* was cut across the bases of paraphyses and spermatiophores. The lower epidermis (*b*, *f*) has been penetrated at three points. Hyphae branching from the paraphyses have pushed down between epidermal cells at *c*, *d*, and *e*, reaching the lower surface of the leaf. The one at *d* may be covered by the cuticle of the leaf, but, so far as can be discerned, the others are not. As in the other rusts studied (*2*, *3*, *4*), hyphae reaching the leaf surface are short-lived. Only the one at *d* is still living.

In the 9-day infection on the older leaf (Table 1) the surface hyphae differ in number, distribution, and condition from those on the younger leaf. Only 25 surface hyphae were found—less than half as many as on the younger leaf. Moreover, all 25 were dead—not one, so far as can be judged by the appearance, was in condition to function.

Typical examples from this 9-day infection have been drawn. Plate 4, A, shows a hypha (*b*) that pushed up between cells of the upper epidermis, then died and shrank back part way. A definite layer of cuticle covers it at *a*. Plate 4, B, shows a bit of the lower epidermis with a short subcuticular hypha (*a*), now dead and inclosed on all sides by a thick layer of cuticle.

Ten of the surface hyphae of this infection (table 1) occurred at the lower surface in stomata. Even more of them attempt to enter stomata and fail. Plate 4, C, shows a stoma (*a*) and above it, at *c*, the large loose cells of the "space-making" part of a young aecium.

EXPLANATORY LEGEND FOR PLATE 4

A.—Detail from upper epidermis of 9-day infection. A hypha (*b*) has grown up to the outer wall (*a*) from subepidermal mycelium and then died. × 1,020.

B.—Detail of lower epidermis from 9-day infection. A portion of a subcuticular hypha is embedded in heavy walls at *a*. × 1,020.

C.—Heavy-walled closed stoma at *a*. Above it at *b* is a hypha unable to enter the stoma; aecial cells at *c*. Nine-day infection. × 1,020.

D.—Longitudinal view of guard cell (*b*, *d*) in epidermis beneath aecium (*a c*). Dead stomatal hypha (*c*). Nine-day infection. × 1,020.

E.—Aecial primordium from 8-day infection showing a dense upper area (*a*); a less dense lower area (*b*) and dead hyphae in the stoma at *c*. × 320.

F.—Detail of aecial primordium (*b*) of a 10-day infection. A living surface hypha in lower epidermis at *a*. × 1,020.

G.—Diagram of section through 10-day fertile infection, showing spermogonium (*b*), external fungous growth (*a*), and young fertile aecium (*c*). × 115.

H.—Detail from G at *a*, enlarged, showing external fungous growth (*a*) connecting with spermogonium at *b*. × 1,020.

The guard cells of the stoma possess thick walls and the stoma is tightly closed. A hypha is pressed into the angle between the guard cells at *b*, but can not effect an entrance. This is of common occurrence. Under these conditions relatively few of the stomata are occupied by hyphae, and when a hypha does enter it soon dies. Plate 4, D, shows a longitudinal section of a guard cell (*b*, *d*) with aecial cells above it (*a*, *e*). A hypha (*c*) grew into the stoma but is now dead.

The earliest aecial primordium noted was in a 6-day infection. Aecial primordia are of rare occurrence, however, during the first week of development of the rust; on the eighth day after inoculation they become more common. Plate 4, E, shows one at a low magnification from an 8-day infection. It consists of a tangle of hyphae in the mesophyll near the lower surface of the leaf. The upper part of the aecial primordium (*a*) consists of small dense cells, and the lower part (*b*) is made up of larger vacuolated cells more loosely spaced. It is gametophytic throughout; the cells are uninucleate. At *c* is a stoma occupied by hyphae, now dead. These are the only surface hyphae adjoining this aecial primordium, and they appear incapable of functioning.

A number of similar aecial primordia have been examined. Some show no surface hyphae in the contiguous epidermis; others have one or even two, either between epidermal cells or in stomata. These surface hyphae often are dead. Occasionally, however, one is living and apparently capable of functioning, as in the example drawn from a 10-day infection in plate 4, F, at *a*.

Aecial primordia (pl. 4, E, *a*, *b*) consist of a loose snarl of hyphae centered a short distance above the lower epidermis. They exert no appreciable pressure on the lower epidermis. Even when somewhat larger and more massive and when the mesophyll cells included within them are partly crushed, the contiguous lower epidermis is not noticeably bulged. By the twelfth day after inoculation, however, the growing potential aecia, whether fertile or sterile, exert marked pressure on the lower epidermis, stretching it and tending to force open the stomata. Moreover, in well-grown 12-day infections the infected leaf tissues are becoming hypertrophied. Palisade and mesophyll cells are expanding rapidly and the infected part of the leaf may have doubled in thickness. This puts additional stress upon the epidermis, which incloses the expanding tissues. At the same time the epidermis, especially the upper epidermis, is becoming tougher and more impenetrable as the leaf matures.

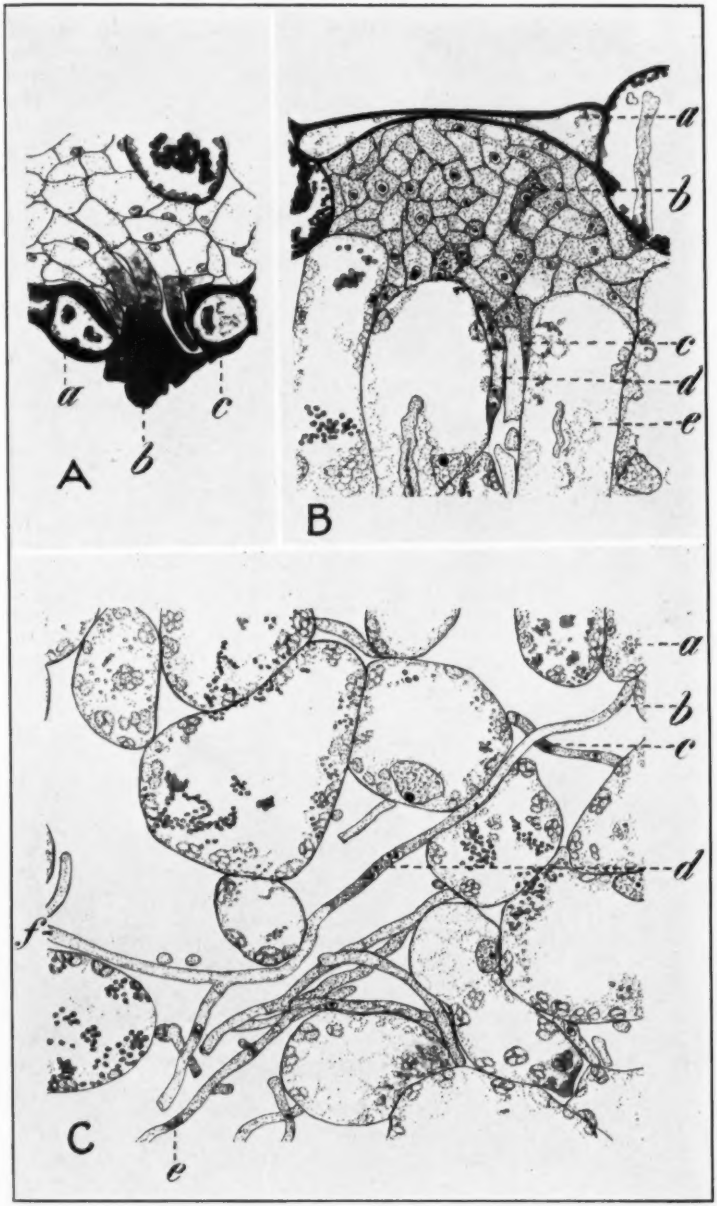
These conditions produce a marked change in the formation of surface hyphae. As the epidermal walls thicken, the hyphae that have pushed out between epidermal cells die and disappear. Steps in this process are seen in plate 4, A and B, and soon after this these hyphae become indistinguishable. The formation of new hyphae between epidermal cells becomes increasingly difficult as the epidermal

EXPLANATORY LEGEND FOR PLATE 5

A.—Detail of 51-day infection, showing the widely separated guard cells of a stoma (*a*, *c*) filled with a group of receptive hyphae (*b*), now dead. × 1,020.

B.—Detail from 10-day infection. Between the flattened upper epidermis (*a*) and the palisade (*e*) is a lateral section through a spermogonium with a trinucleate cell (*b*) among the roots of the paraphyses, and sporophytic hyphae (*c*, *d*) growing downward from them. × 1,020.

C.—Portion of infection below the spermogonium in B, showing lower end of palisade (*a*) a hypha (*b f*) containing 5 nuclei at *d*, and other sporophytic hyphae at *c* and *e*. × 1,020.



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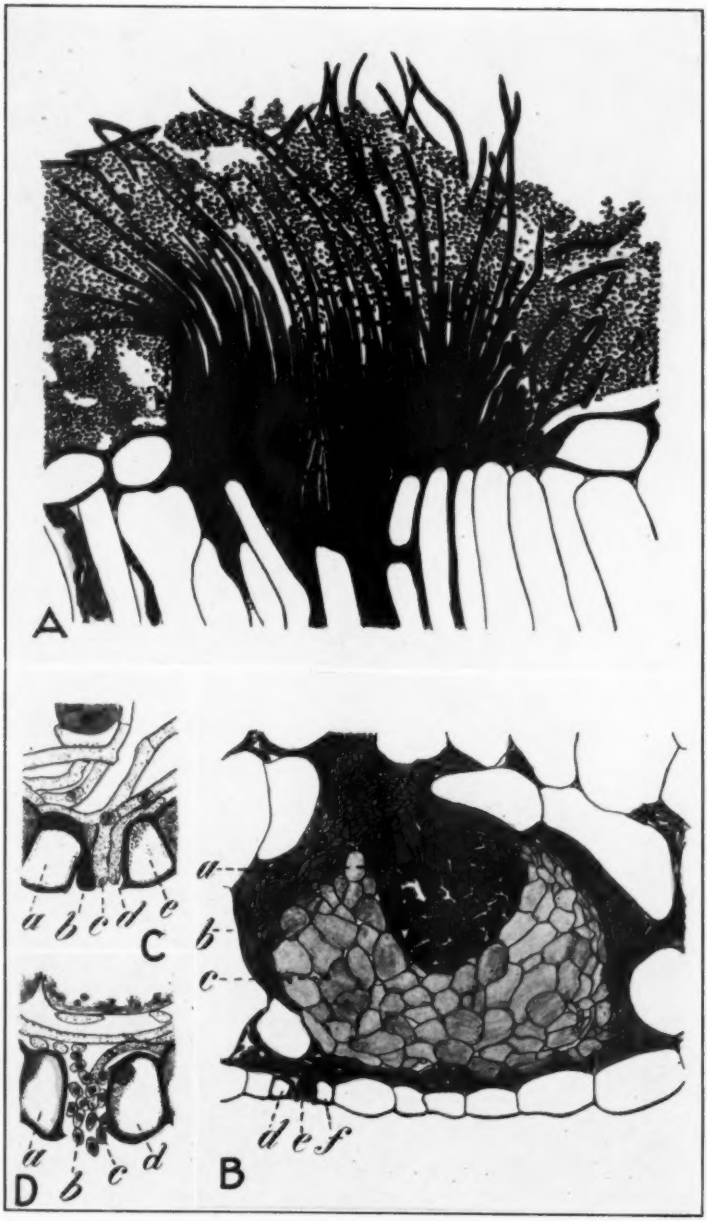

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walls become heavier. One result of this is a rapid decrease in the number of hyphae at the upper surface. At the same time the stomata (which, in the barberry, are on the dorsal surface of the leaf) are under increasing tension and are more easily opened; they soon become crowded with hyphae.

Table 2 shows the results of studies of the number and location of surface hyphae in older sterile infections. There were very few receptive hyphae left on the upper surface and these were in the disturbed areas at the ostioles of spermogonia. Over 95 percent of the surface hyphae were in the lower epidermis, some adjoining aecia, others near spermogonia, and still others in between. Nearly all were in stomata. In the oldest infection studied (51-day), 214 out of the total 220 surface hyphae were in stomata. In many cases a stoma was stretched wide open (pl. 5, A, *a*, *c*) and crowded with half a dozen hyphae (*b*). Nearly all these hyphae were dead, for stomatal hyphae are short-lived.

As noted earlier (*1, p. 598*), in isolated infections in which fertilization is prevented the spermogonia (pycnia) remain active for a surprisingly long period. The drop of spermogonial exudate on the upper surface of the leaf is maintained as long as the fungus lives. These long-lived spermogonia undergo marked secondary changes. The original paraphyses wither and new ones form from deeper parts of the spermogonial wall. Even the spermatiophores may elongate, taking on the appearance of paraphyses, and the whole spermogonium opens out. The extreme case shown in plate 6, A, as compared with the newly matured spermogonium shown in plate 2, E (both of which are drawn at the same magnification), indicates the extent of these secondary changes. In instances where fertilization obtains, whether it takes place early or late, the formation of spermatia ceases, the exudate dries, and the spermogonia die.

TABLE 2.—*Number and location of surface hyphae in older sterile infections of different ages*

Age of infection	Upper epidermis (at spermogonia)	Lower epidermis				Total
		Adjoining aecia	Adjoining spermogonia	Between aecia and spermogonia	In stomata	
14 days	1	25	3	22	41	51
20 days	2	9	31	66	106	108
51 days	1	133	5	81	214	220

There is a relatively small amount of spermogonial exudate on the lower surface of infections—nearly all is on the upper surface, where it would be most easily seen by flying insect visitors. Yet if insects should transfer nectar to the upper surface of older sterile infections, fertilization could not be effected through ordinary surface hyphae,

EXPLANATORY LEGEND FOR PLATE 6

A.—Wide-open, active spermogonium showing marked secondary changes. Fifty-one day infection. × 320.
B.—Fertile aecium from 12-day infection, showing spore chains (*a*), spermogonial wall (*b*), and space-making area (*c*). Below is a wide-open stoma (*d*, *f*) filled with hyphae (*e*). × 320.
C.—Stoma (*a*, *e*) filled with hyphae (*b*, *c*, *d*). Twelve-day infection. × 1,020.
D.—Stoma (*a*, *d*) stomatal hypha (*c*), and spermatia (*b*). Twelve-day infection. × 1,020.

for the upper leaf epidermis has long been impermeable to hyphae. The only way in which fertilization could occur at the upper surface would be through the spermogonia themselves.

On the lower surface, on the contrary, there is a steadily increasing number of stomatal hyphae in aging sterile infections. These presumably could serve to receive spermatial nuclei. It should be added, however, that these stomatal hyphae do not become common until the aecial primordia are past the most favorable stage for the initiation of the sporophyte.

It may be that fertilization can take place at any time when fresh spermatia of one sex are placed in contact with living hyphae of the other sex. In the material at hand, however, it has been rare to find sporophytic mycelium (evidence that fertilization has taken place) before the ninth day after inoculation.

In an earlier paper (*1*) an instance was given suggesting one means of fertilization. In an open spermogonium at the upper surface of the leaf, fungous cells with two and three nuclei were found at the base of the paraphyses (*1, pl. 11, C and D*) and in hyphae leading downward from them. The successive sections of this spermogonium have been reexamined. There is no break in the epidermis around the spermogonium. There are no surface hyphae between epidermal cells anywhere near the spermogonium. The only means of entrance for spermatial nuclei is through the spermogonium itself.

In active spermogonia new paraphyses continue to form; these fresh young paraphyses may serve as receptive hyphae, the spermatial nuclei entering them and migrating down through them to the cells of the spermogonial wall at their base. Further evidence along this line has been found. Plate 5, B, shows a lateral section through a spermogonium that includes a cross section through the bases of paraphyses and spermatiophores. Above it is a much-flattened epidermal cell (*a*) and below it the palisade layer (*e*). At *b*, in the central area of the fungous mass, is a trinucleate cell that has begun to grow downward, and at *c* and *d* are sporophytic hyphae growing down between palisade cells.

The mesophyll tissue just below this spermogonium is shown in plate 5, C. Leading down from the inner end of the palisade cell at *a* is the hypha *b f*, containing a group of five nuclei in its central region at *d*. Appearances here suggest that these nuclei and the cytoplasm about them are migrating down the hypha. Certainly here is no ordinary growth of a sporophytic hypha with regular binucleate cells and a conjugate division at the formation of each new cell. Other sporophytic cells are found at *c* and *e*.

Cases of this sort have been observed repeatedly, where the sporophyte appears to have arisen in a spermogonium and spread from there either by new sporophytic growth or by the migration of nuclei through already existing hyphae.

No unmistakable case has been found in which the sporophyte started in a separate surface hypha between upper epidermal cells. Usually the great majority of these hyphae have died and disappeared by the time fertilization ordinarily takes place. Unless a barberry leaf is exceptionally young when inoculated with the rust, it has made good progress toward maturity by the tenth day following, and its epidermis is nearly or quite impenetrable to any hypha.

Mention has been made of a surface growth of hyphae two cells thick. An example of a surface growth three cells thick is shown in plate 4, G and H. Plate 4, G, is a diagram of a 10-day infection magnified 115 times. At *b* is a slightly lateral section through a mature spermogonium, and at *c* a section through a fertile aecium. At *a* is a close group of hyphae outside of the epidermis. This detail is drawn enlarged as plate 4, H. The fungous growth (*a*) is three cells thick. At *b* it connects through the epidermis with the spermogonial hyphae. This external strand of hyphae runs through a whole row of sections, then forks into two lesser strands which disappear. It is not known whether such a growth as this can serve as an avenue of entrance for spermatial nuclei.

Fertilization had occurred in this infection a day or two before fixation. There were a few binucleate cells in the wall of the spermogonium, in the surrounding mycelium, and scattered all through the aecium (*c*); it was too late to determine, however, whether the spermatial nuclei entered at this external fungous growth or at the paraphyses of the spermogonium.

A count was made of the surface hyphae in three fertilized 10-day infections on the leaf from which the drawings shown in plate 4, G and H, were made. The total number of hyphae at both leaf surfaces for the 3 infections was 65, of which only 10 were unmistakably living. Ten of the sixty-five were at the upper epidermis; 8 between epidermal cells near the ostioles of spermogonia (the most vulnerable place), and only 2 in locations remote from spermogonia. Fifty-five were at the lower surface; 6 at spermogonia, 17 near aecia, and 32 not located at either a spermogonium or an aecium. Comparatively few of these 55 were between ordinary epidermal cells. Nearly all were in stomata, this apparently being the only feasible means of egress, and nearly all were dead. They may have been killed by the closing of the powerful thick-walled guard cells of these little stomata. In fact, a few dead pinched-off bits of hyphae may be seen outside the stoma.

A study was made of another 10-day fertilized infection, which for some unknown reason had not made a good growth but in which a few open spermogonia and young aecia were observable. A careful survey of both upper and lower epidermis revealed not a single receptive hypha, living or dead. This exceptional situation affords valuable confirmation of the hypothesis that spermogonia may serve as avenues of entrance for spermatial nuclei. In this case the spermogonia were the only means of entrance, yet there were occasional binucleate cells in the walls of the spermogonia, in the mycelium, and in at least one of the aecia.

In the fertile infection, as in the sterile, when the aecia grew larger and host tissues became hypertrophied there was a rapid increase in the number of wide-open stomata filled with hyphae. Plate 6, B, shows semidiagrammatically a detail from a 12-day infection. This leaf was exceptionally young when inoculated, as evidenced by the fairly thin walls of the epidermal cells. The fungus grew rapidly in the young tender leaf, forming numerous spermogonia, receptive hyphae, and aecia. Fertilization occurred on the ninth day, three days before the material was fixed. One of the fertile aecia is shown in plate 6, B. The young spore chains at *a* (not a median section) are well established. Below, the stoma (*d*, *f*) is wide open and filled with hyphae

(*e*). There are many such stomata. Surface hyphae have formed at 96 points in this infection, of which 57 are at stomata.

Plate 6, C, shows another of the occupied stomata of this infection. Between the guard cells (*a*, *e*,) are three hyphae. The oldest (*b*) evidently developed while the stoma could still open and shut, for this hypha is pinched in the middle and the part exterior to the constriction is dead. The two younger hyphae in the same stoma (*c*, *d*) show no such constriction and evidently formed after the stoma had become inactive.

A third stoma of this infection (pl. 6, D) is of greater interest, for here, in addition to the single receptive hypha (*c*) in the wide-open stoma (*a*, *d*), there are a dozen or so spermatia at *b*. There is no proof that fertilization occurred at this point, for the adjoining mycelium is gametophytic. In fact, these spermatia may have been produced by this same infection and so may be incapable of fertilizing it. Fertilization, however, could probably take place in this way if spermatia from another infection of opposite sex were placed there. To be sure, stomatal hyphae do not form in any abundance in *Puccinia graminis* until after the first aecia have passed the stage most favorable for the initiation of the sporophyte, but new aecia continue to form for a few days afterwards.

All attempts to find proof of fertilization through stomatal hyphae have failed. This was to be expected. The barberry plants used in this work were grown in the greenhouse. They were protected by cages made of light wooden frames covered by tarlatan to prevent visits from flying insects. Moreover, they were remarkably free from aphids and thrips, which visit the lower surfaces of leaves in other plants. It seemed possible that barberry leaves are too tough for such insects or that some chemical quality of the leaves repels them. Under such circumstances, no fertilization could be brought about by insect carriers of spermogonial exudate. At the time of these experiments (1928) it was not known that fertilization could occur at the lower surface of infected areas. When nectar was transferred during the experiments, it was placed upon the upper leaf surface at infected spots. Fertilization would have occurred at the lower surface only by accident. In view of what is known of other rusts, however (*3*, *4*, *5*), it seems probable that fertilization through stomatal hyphae can occur when conditions are favorable.

DISCUSSION

Heterothallism in *Puccinia graminis* and the function of the spermatia (pycniospores) in effecting fertilization have been adequately demonstrated by the experiments of Craigie (*8*, *9*, *10*, *11*). The ability of the physiologic forms of *P. graminis* to form hybrids on the barberry, resulting in the production of new physiologic forms, has been demonstrated by Waterhouse (*26*), Newton, Johnson, and Brown (*22*, *23*), and Stakman, Levine, and Cotter (*24*).

The means by which spermatia enter remained unknown until Andrus (*5*) discovered in *Uromyces appendiculatus* (Pers.) Fries hyphae which grow to the surface of the leaf and into which spermatial nuclei might enter directly. The initiation of the sporophyte by the entrance of spermatial nuclei into hyphae at the surface of the host tissue is now known for the following rusts: *U. appendiculatus*, *U.*

vignae Barclay (*5*), *Puccinia triticina* Eriks. (*2, 3*), *P. coronata* Cda. (*4*), and *P. graminis*. This method probably will be found to be much more widespread; how much more is, at present, only a matter of conjecture. Andrus cites several cases in which authors have quite incidentally figured or described surface hyphae. The resemblance between Kursanov's drawings of *Aecidium punctatum* Pers. (*17, p. 13*), and plate 2, A, and plate 4, F, of the present paper, is too close to need further comment.

Only half a dozen rusts are known to be heterothallic, but there is little question that heterothallism will be found in many others. There is, however, much adverse evidence. A long series of papers on the cytology of the aecial generation of rusts, published before the discovery of heterothallism and adequately summed up in several papers (*1, 6, 12, 16, 17*), presents evidence that the sporophyte generation is initiated, not at the surface of the host plant but in the sporogenous area of the aecium, by fusions of cells of adjoining hyphae of the same individual or even of two cells of the same hypha. This viewpoint seems incompatible with the results of recent investigations in this field. Only by a detailed study of representative rusts, both long-cycle and short-cycle, from the different rust groups can it be determined whether heterothallism and a method of fertilization through spermatia and receptive hyphae are of general occurrence.

In this connection the remarkably constant presence of spermogonia (pycnia) just before the advent of the sporophyte generation in rusts has been recognized as significant. Jackson (*16, p. 98–99*) notes—

> that the pycnia are quite constantly associated with long-cycled species and that they are omitted most commonly in those which give some evidence of being in an unstable condition as to life history and in short-cycled species or those of reduced life cycle. If the pycniospores exercise the function indicated by Craigie's work, then it would appear quite probable that they would be functionless in homothallic species and might soon be dropped from the life cycle.

He ventures the generalization that—

> the fact that pycnia are so universally present in normal heter- and aut-eu-forms suggests that heterothallism may be a primitive character in the rusts and the usual condition in the present long-cycled species.

The age of the barberry leaf, which serves as host, affects the development of *Puccinia graminis* at more than one point. It had already been known that the entrance of the germinating sporidium may be prevented by the heavy outer epidermal wall of older leaves. Melhus, Durrell, and Kirby state (*21, p. 297–298*):

> When the leaves of *Berberis vulgaris* have taken on a dark green color and crisp, hard appearance, infection apparently does not take place. * * * The marked difference in the susceptibility of young and old leaves of the barberry may be due to the thickness of cuticle and epidermis, considering that infection is accomplished by penetration of the tissues and not through invasion of stomata.

Following up this question, Melander and Craigie (*20*), in studies of the comparative resistance of different species of barberry to stem rust, measured the thickness and resistance to puncture of the outer epidermal walls of leaves of different ages. They found that the species of Berberis whose leaves possess epidermis that is very resistant to puncture usually are resistant to rust, and state (*p. 99*): "But the leaves of susceptible species also become practically immune with age. * * * Leaves of *B. vulgaris* 10 days old are fairly resistant to *P. graminis*."

Other instances are known in which the entrance and progress of a parasite are held in check by the firm texture of the host tissues. Valleau (*25*) and later Willaman, Pervier, and Triebold (*27*) studied the varietal resistance of plums to brown rot and found that resistance was at least partly mechanical. Resistant varieties differ from susceptible varieties in possessing a tougher cuticle, corky walls about the stomatal cavities, higher fiber content, and firmer flesh. Hursh (*15*) describes the sharp limitation placed upon the spread of mycelium of *Puccinia graminis* in the stems of wheat varieties with abundant sclerenchyma. Goulden (*13*) discusses the possibilities of "mature plant resistance" (due to the development of abundant sclerenchyma) in breeding for resistance to *P. graminis*. And Goulden, Newton, and Brown (*14*) report varietal tests of mature-plant resistance looking toward that end.

Less has been said about the possibility that the tough epidermis of a leaf may interfere with the reproductive processes of a parasite already established within it. There must, however, be many cases like that reported by Kusano (*18*). He studied the resistance of Oenothera species to the attack of *Synchytrium fulgens* Schroet. and found that the thick, tough epidermis of *Oenothera odorata* Jacq. often prevented infection, and that when infection did take place the fungus developed normally but that "the mature sorus was sometimes incapable of dehiscing and ultimately turned brown inside the host-cell" (*18, p. 314*).

In *Puccinia graminis*, if the barberry leaf is young when infected, reproduction is unrestricted and tends to be of the type found in the other cereal rusts. If the leaf is somewhat older, the infection grows and spermogonia form and break through the epidermis; but receptive hyphae form with difficulty, and fertilization depends upon the spermogonia or later upon delayed receptive hyphae in old wide-open stomata. If the leaf is still older at the time of inoculation, infection takes place; but the mycelium remains minute, causes little or no hypertrophy of the host, and does not form reproductive structures. If the leaf is very old no infection takes place.

The terminology of the reproductive processes in rusts remains a vexed question. Genetically, as Craigie (*9, 10, 11*) clearly proved, a (+) sporidium develops into a (+) gametophytic mycelium, which bears (+) spermogonia and spermatia, while a (−) sporidium grows into a (−) gametophytic mycelium with (−) spermogonia and spermatia. On that basis, the spermatia of a male individual are male and can function only when transferred to a female infection, whereas the spermatia of a female infection are genetically female and can function only when transferred to a genetically male individual. If there were no other data available, there would be small argument for the use of the words "spermogonium" and "spermatia," as these are names properly applied only to male structures. It is with some hesitation, therefore, that one continues to use the word "spermogonium" for a structure that can and does perform both male and female functions.

Consideration must be given, however, to the discovery by Andrus (*5*) of hyphae that grow to the surface of the leaf and serve to receive spermatial nuclei. If, as Andrus believes, these hyphae grow from "eggs" in the fertile area of young aecia and are to be considered

"trichogynes," there may be at least a morphological reason for the use of male and female names for the reproductive structures, although no genetical basis has been found.

So far, studies of heterothallism in cereal rusts afford little support for a belief in trichogynes. Hyphae grow to the surface of the leaf several days before the first aecial primordium appears. Some of the surface hyphae that form later have no direct connection with the aecia, except in so far as any monosporidial mycelium constitutes a connected system of hyphae. Even in a species in which a direct connection between receptive hyphae and aecia is of common occurrence, as in *Puccinia triticina* (*2*, *3*), the receptive hyphae form in stomata from ordinary mycelium and aecial primordia then develop around them. The sporogenous area of the aecium takes shape a day or two later. In the cereal rusts, therefore, small justification has been found for the use of the words "egg" and "trichogyne."

In general, a single gametophytic mycelium bears both spermogonia and receptive hyphae. In other words, an individual whose cultural data indicate it to be genetically unisexual bears both the cells to be transferred to other infections and the hyphae that will receive cells from other infections. Genetically, it is unisexual; functionally, it is bisexual. In *Puccinia triticina*, however, there is some degree of segregation. Some individuals bear chiefly spermogonia, and others bear chiefly receptive hyphae and aecia. In a former paper the writer (*2*) has stated: "The infections of *Puccinia triticina* can be arranged into a series with an almost exclusively spermogonial type at one end and a completely aecial type at the other." Although it has not been proved to have a genetic foundation, this unequal distribution of spermogonia and receptive hyphae seems to offer added justification for the use of a sex terminology for these organs.

At the other extreme is the condition found in *Puccinia graminis*. When the texture of the leaf permits, receptive hyphae form between epidermal cells in *P. graminis*, as in the other cereal rusts. When the epidermal walls of the leaf become so tough that the hyphae can not force their way out, fertilization, at the upper epidermis at least, can take place only through the spermogonium itself.

From the point or points of its origin on the leaf surface, the sporophyte spreads rapidly through the mycelium and invades the aecial primordia. There is some evidence here, as in the other rusts studied (*2*, *3*, *4*), that the spread of the sporophyte is achieved, at least partly, by nuclear migrations. The group of five nuclei in the hypha in plate 5, C, *d*, is typical. In the earlier work on *Puccinia graminis*, it was found that irregularities in the number, size, and location of nuclei within a cell were common, and that successive cells frequently contained different numbers of nuclei (*1*, *pls. 13, C, and 14, A*). There is no means of watching the actual migration of nuclei, but the observed facts may be explained on the assumption that introduced nuclei divide and migrate through the mycelium. This would bring the rusts into line with the basidiomycetes in which Lehfeldt (*19*) and Buller (*7*) have studied the progressive diploidization of a gametophytic mycelium by the introduction of nuclei from another mycelium, by the division of these introduced nuclei, and by their migration throughout the mycelium.

SUMMARY

The gametophyte generation of *Puccinia graminis* on the barberry leaf produces spermogonia, receptive hyphae, and gametophytic aecia.

When growing in young, tender leaf tissue hyphae push out freely between epidermal cells to both the upper and lower surfaces of the leaf and sometimes grow for a short distance along the surface. Where growing spermogonia at either leaf surface lift and stretch the epidermis and weaken it by tension, surface hyphae are formed in great abundance.

Barberry leaves rapidly become tough and resistant as the yapproach maturity and the outer epidermal walls become thickened. The surface hyphae already formed between epidermal cells die and disappear and new surface hyphae form with increasing difficulty or fail to develop altogether.

All stomata in the barberry leaf are in the lower epidermis. Hyphae in stomata are rare in young infections and those that do form are quickly killed by the closing of the stoma. A stoma, located where a spermogonium lifts and stretches the epidermis, may be forced wide open and soon fills with hyphae.

As the infection grows and the host tissues become hypertrophied and growing aecia add to the volume, the whole epidermis is under increased tension and stomata are easily opened. Wide-open stomata crowded with one to six hyphae become more and more common.

Under the conditions of the present experiments, fertilization commonly takes place at the upper surface through the spermogonia. In some instances this is the only possible means of entrance for spermatia. If fertilization is prevented, the spermogonia remain active as long as the fungus lives and continue to grow and broaden out and form fresh paraphyses, and the drop of spermogonial exudate (spermatia plus a liquid) is maintained. A day or two after fertilization, whether it takes place early or late, the formation of spermatia stops, the exudate dries, and the spermogonia die.

It is probable that fertilization also can occur by means of the stomatal hyphae of the lower surface, although this has not been observed.

The sporophyte spreads from the point or points of its origin by growth of new hyphae or by migration of nuclei through existing hyphae.

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WEEDS AS POSSIBLE CARRIERS OF LEAF ROLL AND RUGOSE MOSAIC OF POTATO[1]

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INTRODUCTION

The host range of certain viruses, such as curly top of sugar beets (*13*),[3] aster yellows (*9*), and cucumber mosaic (*7*), includes many different families of plants. It is known that certain viruses—for example, the latent virus of apparently healthy[4] potatoes (*3*, *6*, *11*, *15*)—may be masked in one host but show definite mottling in other hosts and a necrotic effect in still others. The symptoms of a virus may also appear as a mottling on one host and as at chlorosis when the virus is transferred to a different host. For example, Hoggan (*4*) found that when Johnson's tobacco virus no. 1 was transmitted to *Capsicum annuum* L. a chlorotic condition without mottling was produced.

Rugose mosaic and leaf roll are two of the most important virus diseases of potato (*Solanum tuberosum* L.) in Oregon. The former occurs in all parts of the State, whereas the latter is confined principally to the potato-growing areas east of the Cascade Mountains. In some sections of the State these diseases are difficult to control by roguing. It seems probable that weeds may serve as hosts of these and other virus diseases of potato and may be a source of infection when growing near potato fields.

In the summer of 1929, while inspecting potato fields in central Oregon that showed a large percentage of leaf roll, the writer discovered many plants of nightshade (*Solanum villosum* Mill.). Upon examination it was found that a number of these plants were infected with a disease resembling leaf roll. Infected plants were slightly dwarfed and their leaves were rolled, leathery, and chlorotic, whereas healthy plants in the same field were vigorous and had leaves of a normal green color.

A potato field showing about 50 percent rugose mosaic was found in the same section. Many of the plants displayed typical current-season symptoms of rugose mosaic, namely, necrosis and dropping of the leaves. Nightshade was likewise abundant in this field, but a thorough examination of several hundred plants did not reveal a single one that showed mottling or necrosis. It was therefore thought that this weed might not be a host of rugose mosaic; subsequent experiments have indicated, however, that it is.

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[3] Reference is made by number (italic) to Literature Cited, p. 31.

[4] Apparently healthy potatoes are regarded as healthy in commercial practice, but they harbor in masked condition a virus disease which has been called "latent virus."

A study of the host range of the viruses that affect cultivated plants is of primary importance in formulating a program for their control. Since symptoms of the same virus may vary on different species of plants and even on different varieties of the same species, it is also essential to determine the symptomatology of these virus diseases on the various hosts. In order to learn more about host relationships of potato viruses, the experiments reported in this paper were begun in the fall of 1929.

REVIEW OF LITERATURE

The importance of weeds as hosts of virus diseases of potato has been mentioned in some publications (*10*, *12*, *16*, *17*), but very few specific data have been presented bearing upon the relation of such hosts to the spread of these diseases to potatoes under natural field conditions.

Quanjer (*10*) states that tobacco (*Nicotiana tabacum* L.), *Atropa belladonna* L., *Datura stramonium* L., *Hyoscyamus niger* L., *Solanum nigrum* L., and *S. dulcamara* L., when infected by grafting or by means of aphids, are able to carry leaf roll although they do not show symptoms of it, as is indicated by the fact that the disease can be transmitted back to potatoes by grafting the vines with tops of such infection carriers. The species of aphids used and the percentage of infection secured are not given. The writer (*2*) has transmitted leaf roll by means of *Myzus persicae* (Sulz.) from potato to tomato, pepper, *Datura stramonium*, *D. tatula* L., *S. nigrum* [5] and *S. dulcamara*.

Quanjer's crinkle, as pointed out by Johnson (*7*), is probably identical with Schultz and Folsom's (*12*) rugose mosaic. Quanjer claims to have transmitted crinkle by grafting to tomato (*Lycopersicon esculentum* Mill.), *Solanum nigrum*, *Datura stramonium*, *Atropa belladonna*, and *Hyoscyamus niger*. He states that the symptoms resembled those of potato, but makes no reference to return inoculation to potato.

Schultz and Folsom (*12*) found that the tomato is susceptible to mild mosaic and rugose mosaic. Young and Morris (*17*) transmitted rugose mosaic from potato to tomato. Fernow (*3*) secured but one disease when he transferred juice from diseased or healthy potatoes to other solanaceous plants. He referred to this as virus B. Blodgett (*1*) attempted to inoculate pepper plants with the virus of yellow-dwarf disease of potatoes and also with inoculum from apparently healthy potatoes. All the peppers inoculated from potato, either from the yellow-dwarf tubers or from the healthy tubers, showed the same type of symptom, namely, a severe necrosis of the leaves and stems. He does not state whether return inoculations to potato were made from peppers inoculated with yellow dwarf. Johnson (*5*) found that "healthy" potato virus and vein banding from tobacco, when inoculated into pepper plants, sometimes produced chlorotic rings and necrosis, followed by defoliation. These symptoms never developed from the "healthy" potato virus alone, and the vein-banding virus was not recovered when return inoculations were made to tobacco. Johnson (*6*) was the first to demonstrate the presence of one or several viruses in practically all so-called healthy commercial American

[5] A later and more accurate determination of this species establishes it as *Solanum villosum*. The two species are decidedly similar, but *S. villosum* is the more hairy of the two and its calyx lobes are broad and triangular and united for half their length, whereas *S. nigrum* is glabrous and its calyx lobes are narrow and separate almost to the base.

varieties of potatoes. Valleau (*15*) reported similar results. Schultz (*11*) found that when certain apparently healthy potato varieties were tuber-grafted with each other and with seedlings, necrotic streaks developed on some of the varieties and seedlings.

Johnson (*5*) refers to a disease occurring naturally on tobacco as "vein banding," on account of the narrow bands of dark-green tissue that appear along the veins of the leaves of affected plants. He transferred this virus from tobacco to tomato, petunia (*Petunia hybrida* Vilm.), and species of *Physalis*. Valleau and Johnson (*16*) transmitted the vein-banding virus from tobacco to apparently healthy young Irish Cobbler potato plants and produced typical rugose-mosaic symptoms. When the vein-banding virus was transmitted to potato seedlings free from the latent virus of healthy potatoes, it caused a disease characterized by nearly normal color, faint mottling, rugoseness, and only slight distortion of the leaves.

Smith (*14*) found that when juice of mosaic-affected potatoes was pricked into healthy tobacco plants a ring spot developed. Transmission by the aphid *Myzus persicae* of a virus from mosaic potatoes to tobacco resulted in vein banding. When juice from healthy potatoes was transmitted no symptoms appeared. Koch (*8*) found that the rugose mosaic of potato consists of two distinct viruses. One is easily transmitted by aphids; the other, the "mottle," or latent virus of apparently healthy potato, can be transmitted mechanically but not by aphids. The aphid-transmitted or vein-banding virus, when transferred to tobacco, caused only a clearing of the veins. When transmitted mechanically, the combined rugose-mosaic viruses (vein-banding virus plus latent virus) produced spot necrosis on tobacco.

MATERIALS AND METHODS

The following plants were grown from seed and studied as hosts of virus diseases of potato: jimsonweed (*Datura stramonium* and *D. tatula*), nightshade (*Solanum villosum*), bitter nightshade (*S. dulcamara*), groundcherry (*Physalis* sp.), tomato (*Lycopersicon esculentum*), redpepper (*Capsicum annuum* L.), and petunia (*Petunia hybrida*).

Tubers known to be infected with rugose mosaic or leaf roll and also those from apparently healthy potatoes were planted in pots in the greenhouse. When potatoes infected with rugose mosaic or so-called healthy potatoes served as the source of inoculum, the leaf-mutilation method was used; that is, the leaves from the diseased plants were macerated and the extracted juice was rubbed into the leaves of healthy plants. Previous studies had shown that the incubation period of rugose mosaic is from 3 to 4 weeks; therefore 6 weeks after the plants were inoculated, juice was extracted from their leaves and rubbed into the leaves of a series of healthy potato plants. Potato plants containing the latent virus will manifest necrotic spots on the leaves if they are inoculated by leaf mutilation with the vein-banding virus (fig. 1).

In the spring of 1931, aphids (*Myzus persicae*) were colonized on a Burbank potato plant affected with rugose mosaic and were then transferred to three Marglobe tomato plants. No symptoms developed except a faint clearing of the veins, nor did these tomato plants appear to be stunted in their growth. This species of aphid

does not transfer the latent virus of apparently healthy potatoes, as was determined in tests including nearly 100 plants; it transmits only the vein-banding component of the rugose mosaic. This aphid-transmitted virus has been transferred mechanically by leaf mutilation from tomato to apparently healthy potatoes, potato seedlings, and other solanaceous plants. Valleau and Johnson (*16*) found this virus occurring naturally on tobacco and referred to the disease as vein banding. In the present paper the term "vein banding" is used for the aphid-transmitted component of rugose mosaic.

FIGURE 1.—American Giant (Pride of Wisconsin) potato plant containing the latent virus; inoculated by leaf mutilation with vein-banding mosaic from an infected potato plant; shows necrotic spots on leaves.

The aphid-transmission method was used to transmit leaf roll, since it cannot be transmitted by leaf mutilation. Aphids (*Myzus persicae*) were colonized under muslin-covered insect cages on potato plants infected with leaf roll and left there for 2 weeks or more. About 20 of the aphids were then transferred to each of the plants that were to be inoculated and left there for 6 days. The cages were then removed and the plants were fumigated to kill the aphids. About 20 to 25 days after inoculation, when symptoms of leaf roll appeared, nonviruliferous aphids were colonized on these plants and later transferred to young healthy potato plants, which were also fumigated after 6 days.

The greenhouse was fumigated frequently. Numerous potato plants and other solanaceous plants were growing in the greenhouse, but not a single case of leaf roll developed on any of the uninoculated plants.

The aphids used were obtained from carnation plants growing in one of the greenhouses and were colonized on cabbage plants and egg-

plants growing under muslin-covered insect cages. Occasionally these aphids were transferred to potato plants to determine whether the aphids were free from the leaf-roll virus; in all cases the potato plants remained healthy.

SYMPTOMS OF RUGOSE MOSAIC, VEIN BANDING, AND LEAF ROLL ON VARIOUS PLANTS

SYMPTOMS ON JIMSONWEED

Two species of jimsonweed (*Datura stramonium* and *D. tatula*) developed a pronounced mottling when inoculated by leaf mutilation with juice from apparently healthy potato plants or from potato plants affected with rugose mosaic. The mottling was characterized by large yellowish-green blotches on the leaves.

Attempts were made to transmit the rugose mosaic by leaf mutilation from either species of *Datura* inoculated with this disease to potato plants, but all results were negative (table 1).

TABLE 1.—*Cross inoculations with rugose mosaic from potato by leaf mutilation*

Species tested	Plants inoculated with rugose mosaic from potato		Return inoculations to potato plants		
	Total	Infected	Total	Infections	Notes
	Number	*Number*	*Number*	*Number*	
Solanum villosum	10	7	15	13	Latent-virus mottling.
Physalis sp.	10	6	10	8	Latent-virus mottling and some rugosity.
Petunia hybrida	10	7	(a)		
Lycopersicon esculentum	25	25	30	27	
Datura tatula	10	0	10	0	Latent-virus mottling.
Datura stramonium	10	0	10	0	Do.
Capsicum annuum	15	0	10	0	Necrosis, like that caused by the latent virus.
Solanum dulcamara	10	0	10	0	No symptoms.

[a] No return inoculations to potato.

The vein-banding mosaic failed to produce any symptoms when introduced into jimsonweed, nor could the virus be recovered by making return inoculations into young potato plants (table 2). Valleau and Johnson (*16*) reported similar results.

TABLE 2.—*Cross inoculations of aphid-transmitted vein-banding component of rugose mosaic by means of leaf mutilation*

Species tested	Plants inoculated with vein banding from tomato		Return inoculations to potato plants	
	Total	Infected	Total	Infections
	Number	*Number*	*Number*	*Number*
Solanum tuberosum (seedling)	20	17	(a)	
Solanum tuberosum	20	18	(a)	
Solanum villosum	10	10	6	6
Physalis sp.	10	7	10	7
Lycopersicon esculentum	15	15	(a)	
Datura stramonium	10	0	6	0
Petunia hybrida	10	8	6	6

[a] No return inoculations to potato.

Datura does not appear to be a host of the vein-banding component of rugose mosaic, but it is very susceptible to the latent virus of apparently healthy potatoes. In the summer of 1930, 6 plants of *Datura stramonium* and 6 of *D. tatula* were planted in a potato field, and every one of these weeds developed a pronounced mottling as a result of natural transmission of the latent virus from apparently healthy potato plants. It was not determined whether this transmission was due to insect transfer or to other means, such as the processes of cultivation or mechanical contact of the leaves caused by the wind.

Leaf roll was transmitted by aphids from potato to *Datura stramonium* and *D. tatula* and from these weeds to potato (table 3). On the two species of *Datura*, leaf roll was characterized by a distinct chlorosis

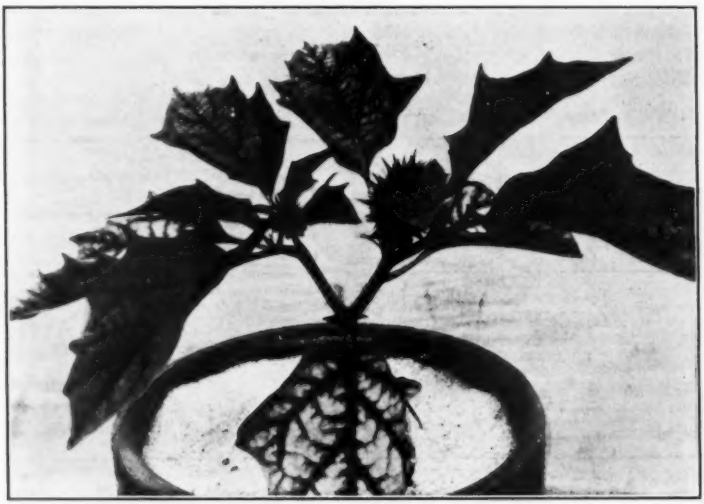

FIGURE 2.—*Datura stramonium* infected with leaf roll through the agency of aphids (*Myzus persicae*) that were transferred from an infected potato plant; shows chlorosis and rolling of leaves.

and rolling of the leaves, which became leathery as the disease progressed (fig. 2).

TABLE 3.—*Cross inoculations with leaf roll by means of aphids (Myzus persicae)*

Source of inoculum	Species tested	Plants inoculated with leaf roll		Return inoculations to potato	
		Total	Infected	Total	Infections
		Number	*Number*	*Number*	*Number*
Potato	*Datura tatula*	5	5	4	3
	Datura stramonium	10	9	10	7
	Solanum villosum	12	10	7	6
	Solanum dulcamara	9	(a)	6	5
	Capsicum annuum	5	4	8	[b] 3
	Lycopersicon esculentum	15	13	9	7
	Physalis sp.	10	0	10	0
	Solanum tuberosum	11	10	----	----
Datura tatula	*Lycopersicon esculentum*	4	3	(c)	----
Tomato	*Solanum villosum*	6	5	(c)	----
	Datura stramonium	2	2	(c)	----
	Lycopersicon esculentum	6	5	(c)	----

[a] Apparently symptomless carrier of leaf roll.
[b] Symptoms not typical of leaf roll.
[c] No return inoculations to potato.

SYMPTOMS ON NIGHTSHADE

Nightshade (*Solanum villosum*) developed a pronounced mottling when inoculated with juice from apparently healthy potato plants or from those infected with rugose mosaic. When infected plants were growing in the greenhouse or under muslin-covered insect cages, the mottling was quite distinct. In the summer of 1930 three nightshade plants infected with rugose mosaic were planted outdoors, but during the summer the symptoms were completely masked and it was impossible to recognize the disease on the plants. However, rugose mosaic was readily transmitted from infected *Solanum villosum* to potato by leaf mutilation (table 1).

Vein-banding virus alone caused a faint vein banding in some of the nightshade leaves, but the symptoms were generally difficult

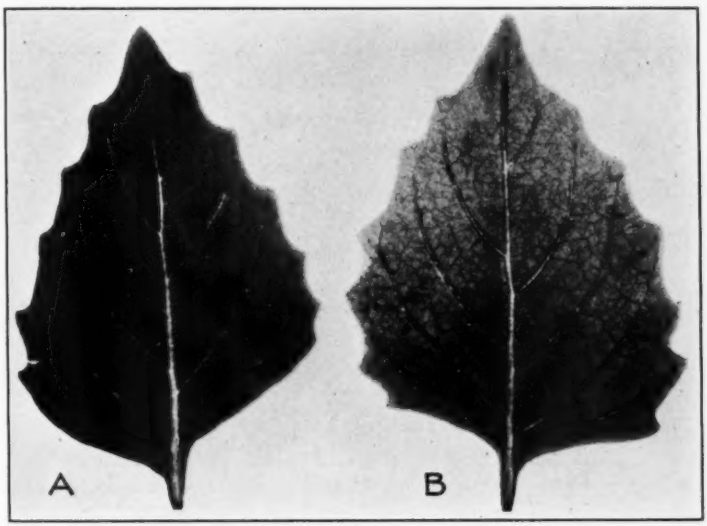

FIGURE 3.—Leaves of *Solanum villosum:* A, Healthy leaf; B, leaf from plant inoculated by leaf mutilation with vein-banding mosaic from an infected tomato plant, showing dark bands along veins characteristic of the disease.

to detect (fig. 3). The virus from such infected weeds can be readily transferred by leaf mutilation to apparently healthy potatoes, causing typical rugose-mosaic symptoms (table 2).

The leaf-roll virus was readily transmitted from potato to nightshade and returned to potato by *Myzus persicae*. The symptoms on nightshade were distinct chlorosis and rolled leaves (fig. 4). The disease was first apparent in some of the younger leaves, which became leathery as the disease progressed; finally all leaves of the plant showed leaf-roll symptoms (table 3).

SYMPTOMS ON BITTER NIGHTSHADE

Bitter-nightshade plants (*Solanum dulcamara*) were inoculated with juice from healthy potato plants and also from potato plants affected with rugose mosaic, but no symptoms of any kind developed, nor

was it possible to recover the rugose-mosaic virus when return inoculations were made to potato (table 1).

When leaf roll is transferred by aphids from potato to bitter-nightshade plants the disease may show very strikingly. One plant to which the disease was transmitted ceased growing and became very much stunted, although it remained alive. The leaves became chlorotic and stiff. Other bitter-nightshade plants inoculated at the same time in a similar manner, although carrying the virus, appeared healthy. Leaf roll was transmitted from both types of plant back to potato (table 3).

SYMPTOMS ON TOMATO

When a tomato plant (*Lycopersicon esculentum*) was inoculated with juice from an apparently healthy potato plant, a faint mottling of pale-green areas developed which was distributed irregularly

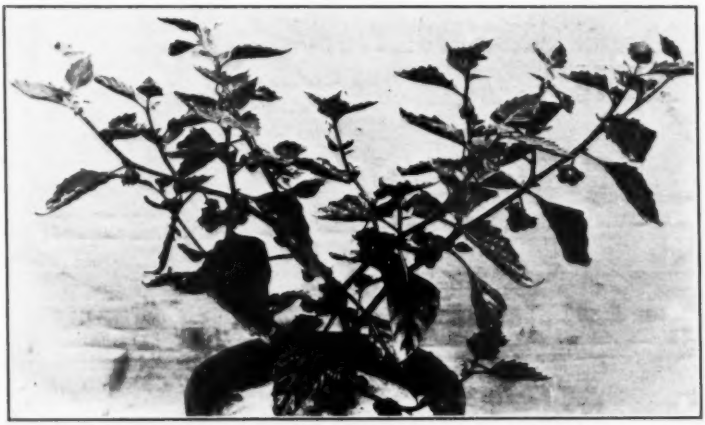

FIGURE 4.—*Solanum villosum* infected with leaf roll through the agency of aphids (*Myzus persicae*) that were transferred from an infected potato plant; shows chlorosis and rolling of leaves.

over the surface of the leaf. Inoculations from potatoes infected with rugose mosaic generally caused a necrosis of the leaves. This disease was readily transmitted from infected tomato plants back to potato. In some cases, instead of a necrosis, mottling developed that was indistinguishable from the type caused by the latent virus. Three tomato plants showing such symptoms were grown in the field under cages during the summer of 1930, but they did not develop necrosis. When juice from these plants was inoculated into potato, however, typical symptoms of rugose mosaic resulted (table 1).

In the spring of 1931, aphids (*Myzus persicae*) from a Burbank potato plant infected with rugose mosaic were transferred to three Marglobe tomato plants. Six weeks after inoculation the tomato plants appeared healthy except for a faint clearing of the veins. Return inoculations by leaf mutilation from each of the three plants into potato plants already carrying the latent virus resulted in typical rugose-mosaic symptoms. This indicated that the aphids had

transmitted the vein-banding component of rugose-mosaic virus to each of the three tomato plants, causing only a slight clearing of the veins and apparently not interfering with normal growth. As previously indicated, in the transfer of leaf roll from potato to solanaceous weeds by *M. persicae* where only leaf roll and not the latent virus was transmitted, this species of aphid is unable to transmit the latent virus.

When the vein-banding virus was transferred by leaf mutilation from tomato to potato seedlings and to plants raised from tubers from second-year seedlings, only an interveinal type of mottling developed, without necrosis or rugosity. These plants remained vigorous for a long time and their growth did not appear to be appreciably checked by the disease. However, when potato seedlings were inoculated by leaf mutilation with the latent virus of apparently healthy potatoes, a severe necrosis of leaves and stems developed, causing the death of the plants 16 days after inoculation (fig. 5).

FIGURE 5.—Potato seedling showing necrosis on leaflets 12 days after inoculation by leaf mutilation with the latent virus from an apparently healthy potato plant.

Leaf roll was also readily transferred from potato to tomato by aphids (table 3). The symptoms were not very distinct. The leaves showed practically no rolling but became rigid and somewhat leathery; no stunting of the plant was observed. Two tomato plants infected with leaf roll were inoculated with tomato mosaic, and although the latent virus was not present, the combination of the leaf-roll virus and tomato mosaic developed typical streak.

Leaf roll was readily transmitted from tomato to potato by means of aphids (fig. 6); it has also been transferred from tomato to jimsonweed and nightshade in the same way.

SYMPTOMS ON GROUNDCHERRY

When the common groundcherry (*Physalis* sp.) was inoculated by leaf mutilation with the latent virus from an apparently healthy potato plant, a faint mottling developed. Inoculation with virus from potatoes infected with rugose mosaic caused a more pronounced mottling and some rugosity, but apparently no necrosis (fig. 7).

Rugose mosaic was also readily transferred from infected *Physalis* to potato (table 1).

When groundcherry plants were inoculated with the vein-banding virus, no symptoms developed; 5 weeks after inoculation, however,

FIGURE 6.—American Grant (Pride of Wisconsin) potato plant infected with leaf roll through the agency of aphids (*Myzus persicae*) that were transferred from an infected tomato plant.

when inoculum from a number of the inoculated plants was transferred to potato plants infected with latent virus, typical rugose-mosaic symptoms developed.

No symptoms were evident after *Physalis* plants were inoculated by means of aphids with leaf roll from potato, nor could the disease be retransferred from such inoculated plants to potato (table 3).

SYMPTOMS ON REDPEPPER

When young redpepper plants (*Capsicum annuum*) were inoculated by leaf mutilation with the latent virus from apparently healthy potato plants or the juice from those infected with rugose mosaic, severe necrosis and leaf dropping resulted (table 1). Apparently there were no differences in the symptoms caused by the two viruses. Transfers to potato of juice from peppers inoculated with rugose

FIGURE 7.—Groundcherry (*Physalis* sp.) showing mottling and rugosity of the leaves produced by inoculating the plant by leaf mutilation with juice from a potato plant infected with rugose mosaic.

mosaic, however, failed to produce any symptoms. No inoculations were made with the vein-banding virus alone.

During the summer of 1930 eight pepper plants were planted in a potato field, but no necrosis or leaf dropping due to probable natural transmission from the potatoes developed.

Attempts were made to transfer leaf roll by means of aphids from potato to pepper. Some rolling and chlorosis developed in the affected plants. When the disease was transferred back to potato some rolling developed, but the symptoms were not typical of leaf roll. Further testing is needed before pepper can be classified as a host of leaf roll (table 3).

SYMPTOMS ON PETUNIA

Plants of petunia (*Petunia hybrida*) were inoculated by leaf mutilation with rugose mosaic from potatoes (fig. 8). Of the 10 plants inoculated, only 1 developed severe necrosis; the other 9 showed no symptoms of disease. No return inoculations from these plants to potato were made. Later inoculations with rugose mosaic have resulted in a mottling or a necrosis of the petunia leaves in a very large percentage of the plants inoculated.

When the vein-banding virus was inoculated into petunia, a faint clearing of the veins developed. After the plants became older these

FIGURE 8.—Petunia showing necrosis on some of the leaves as a result of inoculation by leaf mutilation with juice from a potato plant infected with rugose mosaic.

symptoms disappeared. Return inoculations from these plants to potatoes carrying the latent virus developed typical rugose-mosaic symptoms.

No attempt was made to transmit leaf roll to this host.

TRANSMISSION OF LEAF ROLL AND RUGOSE MOSAIC BY INSECTS OCCURRING ON POTATO PLANTS

It was considered important to determine whether insects that feed on potato plants under natural conditions are able to transmit virus diseases from infected weeds to potato. Accordingly two large muslin-covered insect cages were built. In the larger cage the following plants infected with leaf roll were interplanted among 85 healthy potato plants: *Solanum villosum*, 6 plants; *Datura stramonium*, 4 plants; tomato, 1 plant. Insects collected from a field of healthy

potatoes at the Oregon Agricultural Experiment Station at Corvallis, Oreg., were introduced into the cage. During the growing season 11 percent of the potato plants developed current-season symptoms of leaf roll. At digging time the progenies of all the potato plants were saved, and in the spring of 1931 they were planted in the field. Of the 433 tubers planted, 355 (82 percent) developed into plants showing typical tuber-perpetuated leaf-roll symptoms.

In the smaller cage, 6 *Solanum villosum* plants and 2 tomato plants, all of which were infected with rugose mosaic, were planted among 52 healthy potato plants. Miscellaneous insects, collected from the same field of healthy potatoes as were the insects used to transmit leaf roll, were introduced into the cage in order to determine whether they would spread rugose mosaic from the weeds to the potato plants.

During the growing season 10 percent of the potato plants developed current-season symptoms of rugose mosaic. The progenies from all the plants were saved and planted in the field in 1931. Of the 157 tubers planted, 84 (53 percent) developed typical tuber-perpetuated rugose mosaic; but in addition to this, 9 plants (6 percent) developed tuber-perpetuated leaf-roll symptoms. Since the smaller cage did not have a double door like that built in the larger one, and since the two cages adjoined each other, there is a possibility that some insects from the larger cage were accidentally introduced into the smaller one. No leaf roll was present in the field from which the insects were collected, nor were any current-season symptoms of leaf roll evident in the plants of the smaller cage during 1930.

Specimens of the insects used in these transmission experiments were sent for identification to H. Morrison, of the Bureau of Entomology, United States Department of Agriculture. The following species were identified: *Myzus persicae*, *Illinoia solanifolii* (Ashm.), *Epitrix subcrinita* Lec., *Sciara* sp., *Chironomus* sp., *Empoasca* sp., cicadellid species, *Nabis alternatus* Parsh., *Lygus pratensis* (L.), and *Philaenus spumarius* Fall.

Experiments are now in progress to determine the ability of each of these insects to transmit the virus diseases of the potato.

ATTEMPTS TO TRANSMIT THE LATENT VIRUS BY MEANS OF INSECTS

It is of interest to know whether or not the latent virus of apparently healthy potatoes is readily transmitted by insects. This information is of importance in determining whether promising new potato seedlings should be grown in isolation from apparently healthy potatoes in order to keep them from becoming infected with the latent virus. This knowledge is also valuable in the testing of potato seedlings for resistance to virus diseases. The symptoms of some of these diseases on potatoes free from the latent virus are very mild, and this may erroneously be interpreted as resistance or tolerance of the potatoes to virus diseases.

In order to secure some data on insect transmission of the latent virus, five different species of insects occurring on potato, namely, western potato flea beetle (*Epitrix subcrinita*), leaf hopper (*Empoasca* sp.), tarnished plant bug (*Lygus pratensis*), spittle bug (*Philaenus spumarius*), and *Nabis alternatus*, were collected from a field of healthy potatoes.

In the late summer of 1931, 8 to 10 insects from each of these 5 species were transferred to 5 separate lots, respectively, consisting of 12 individually caged jimsonweed plants. The insects were left on the plants for 7 days; the plants were then fumigated to kill the insects, and the cages were removed. In all cases the jimsonweeds remained normal, with the exception of those to which the spittle bug (*Philaenus spumarius*) had been transferred. These 12 plants developed large yellow blotches on the leaves about 12 days after the insects had been transferred to them. The type of mottling was not quite typical of that caused by the latent virus.

To determine whether the mottling was due to the latent virus or to an enzyme or a toxin injected by the insect, this same species of insect was collected from an alfalfa field and transferred to 10 tomato plants and 10 jimsonweeds. No symptoms developed on the tomato plants, but some blotches were evident on the jimsonweeds about 12 days after the spittle bugs had been transferred to them.

Additional spittle bugs (*Philaenus spumarius*) were collected from a potato field and transferred to 10 tomato plants, none of which developed any symptoms.

No symptoms developed when 10 tomato plants were inoculated by leaf mutilation with juice from jimsonweeds that showed yellow blotches on the leaves after spittle bugs collected from potato plants had fed on them.

These experiments will have to be repeated on a larger scale, and other insects occurring on potato will have to be tested, before any final conclusions can be drawn; but the results secured so far do not indicate that the latent virus of apparently healthy potatoes is readily transmitted by insects.

DISCUSSION AND CONCLUSIONS

It has been demonstrated that certain weeds growing in or near potato fields may, under natural conditions, become infected with potato-virus diseases. Insects naturally occurring on potato plants are able to transmit leaf roll and rugose mosaic from weeds to potato.

When solanaceous weeds become naturally infected with rugose mosaic, only the vein-banding virus may be present; the symptoms caused by this virus on weeds are very difficult to detect.

The fact that vein banding caused only mild mottling on potatoes free from the latent virus, in contrast to the severe disease it develops on apparently healthy potatoes carrying the latent virus, suggests that the effects of certain potato-virus diseases, namely, vein banding and leaf roll, may be greatly intensified by the presence of the latent virus.

Potato seedlings that were inoculated with leaf roll remained alive and vigorous in pots in the greenhouse considerably longer than did apparently healthy potato plants carrying the latent virus that were inoculated with leaf roll.

Weeds infected with leaf roll generally show chlorosis, rolling of the leaves, and a tendency to be leathery.

SUMMARY

Rugose mosaic was transmitted by leaf mutilation from potato to *Solanum villosum*, *Physalis* sp., tomato, and petunia.

Aphids (*Myzus persicae*) did not transmit the latent virus from apparently healthy potatoes to other solanaceous plants.

From potatoes infected with rugose mosaic, *Myzus persicae* transmitted to tomatoes only the vein-banding component of the virus complex.

The vein-banding virus alone causes very faint symptoms in susceptible host plants, characterized in soft-leaved plants by a banding of the veins.

The vein-banding virus when inoculated into apparently healthy potato plants (carrying the latent virus) causes typical rugose-mosaic symptoms; when inoculated into potato seedlings free from the latent virus, vein banding causes only a faint type of mottling.

Leaf roll was transmitted by *Myzus persicae* from potato to *Solanum villosum*, *S. dulcamara*, *Datura stramonium*, *D. tatula*, and *Lycopersicon esculentum*.

Insects naturally occurring on potato can transmit leaf roll and rugose mosaic from infected weeds to potato.

Five different species of insects naturally occurring on potato failed to transmit the latent virus of apparently healthy potatoes to jimsonweed.

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EFFECTS OF PLANT SPACING AND IRRIGATION ON NUMBER OF LOCKS IN COTTON BOLLS[1]

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INTRODUCTION

Practically all the bolls produced by American upland varieties of cotton (*Gossypium hirsutum* L.) have either four or five locks or carpels. Some varieties produce a greater proportion of 5-lock bolls than others, and there is sometimes considerable variation among individual plants of the same variety. The factors that determine the ratio of the 4-lock to the 5-lock bolls are obscure, but are generally considered to be heritable. It is clear, however, that the proportion is materially affected by the conditions under which the plants are grown, such as infertile or alkaline soil; an insufficient supply of moisture; extremes of temperature, sometimes accompanied by a reduction of atmospheric humidity; crowding of the plants in the row; the presence of weeds; and any other conditions that limit optimum development.

When grown under good conditions the Acala variety of upland cotton produces from 75 to 85 percent of 5-lock bolls. The majority of these are set during the middle part of the growing season, after the plants have attained some size but before the general slackening of growth toward the end of the season. In the earlier part of the season, and again toward the latter part, the proportion of 4-lock bolls is often increased and may equal and sometimes exceed the number of 5-lock bolls. Accordingly, it is frequently found that the 4-lock bolls are borne at the inner nodes of the lower fruiting branches and on the outer nodes of the upper branches, although this habit is not constant. Vegetative branches, as a rule, produce a higher percentage of 4-lock bolls than do the fruiting branches.

The number of locks is probably determined very early in the embryonic stage of the boll. At this stage, apparently, the heritable factor that normally determines the number of locks is influenced by physiological conditions in the plant, induced by the conditions under which the plant has been growing. Just when or how these effects are brought about is beyond the scope of this paper. The evident effects of certain environmental conditions on the ratio of 4-lock to 5-lock bolls have been noted, however, and are here discussed.

REVIEW OF LITERATURE

In the study of the morphology of the cotton plant the lock number has received considerably less attention than some other charac-

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[2] The writers are indebted to T. H. Kearney, Division of Egyptian Cotton Breeding, for permission to use unpublished data.

ters, and the literature on the subject is limited. The effect of changed growth conditions was noted in Texas in 1908 by the late Rowland M. Meade, as reported by Cook (*3*),[3] in a planting of Kekchi cotton, a variety native to Guatemala, which normally has a large proportion of 5-lock bolls. Plants from newly imported seed of this variety and from seed of an acclimatized strain, grown near together, showed striking differences, including a greatly decreased proportion of 5-lock bolls in the plants grown from the newly imported seed.

Cook further discussed the effect of changed environment on this character of the cotton plant in 1913 (*4*). Data are given from experimental plantings of Kekchi cotton in 10 localities in California, showing different proportions of lock numbers in the bolls, with the general observation that "drought or other adverse conditions that reduce the crop also diminish the proportion of 5-locked bolls."

In comparing plants spaced at about 2 feet with spacings of 6 to 8 inches in the row at San Antonio, Tex., in 1914, Meade (*9, p. 13*) found that "the range in percentage of 5-locked bolls in wide-spaced rows was from 47 to 72, with an average of 56, while in single-stalk rows the range was from 43 to 61 percent, with an average of 46 percent."

Ballard and Simpson (*1*), working at San Antonio, found on cotton plants whose growth had been retarded by Johnson grass a consistently higher percentage of 4-lock bolls than on plants free from grass in another part of the field.

Although it is generally known that some varieties of upland cotton produce more 5-lock bolls than others, comparatively little has been published on the heritability of the lock-number character in this or other species of cotton. Working in Arizona with Pima Egyptian, normally a 3-lock variety, Kearney (*8*) found that in two selections having relatively high percentages of 4-lock bolls this character was inherited by the progenies. A cross between these strains, in the fourth hybrid generation in 1929, produced 9.1 percent of 4-lock bolls, in contrast with four normally 3-lock inbred strains, which averaged only 3.4 percent of 4-lock bolls.[4]

Harland (*7, p. 159*), working in the West Indies with sea-island cotton, which is normally 3 lock, found that "by selection the percentage of 4-locked bolls has been increased from 20 (the value for ordinary sea island) to 60 or 70." Dunlavy (*6, p. 445*) states:

> Previous work with Acala cotton tends to indicate that the five-lock boll is from ten to fifteen percent larger than the "four" and that the relationship between fours and fives is a heritable character, though greatly influenced by environmental conditions.

There is a well-established preference among farmers for a variety or strain of cotton which produces a large proportion of 5-lock bolls. Since the 5-lock bolls are larger they are supposed to produce more cotton, and in most cases they do, though varieties having a large proportion of 4-lock bolls may yield well. Tyler (*10*) and Dunlavy (*5*) have reported comparative weights of 4-lock and 5-lock bolls in several varieties of upland cotton.

[3] Reference is made by number (italic) to Literature Cited, p. 52.
[4] Unpublished data.

EFFECT OF PLANT SPACING ON NUMBER OF LOCKS

EXPERIMENTS IN 1926

The 1926 cotton-spacing experiments at the United States Acclimatization Field Station, State College, N.Mex., consisted of comparisons, in alternate 4-row blocks, of 2 plants per foot, a so-called "blocked-out" spacing, and unthinned plants, with a standard spacing of 12 inches, or 1 plant to each foot of row. In the 2-plant spacing, the plants were thinned to 2 per hill, with the hills approximately 1 foot apart. In the blocked-out method, the plants were chopped out with an 8-inch hoe, leaving small blocks of plants approximately 8 inches long separated by intervals of similar length. In the unthinned rows the plants grew under crowded conditions, with sometimes as many as 7 or 8 plants per foot, in contrast with the 12-inch-spaced rows, where the plants approximated 1 per foot.

In the fall of the year, when small samples of 4-lock and 5-lock bolls were being picked for laboratory use, it was noted that the requisite number of 4-lock bolls was more readily obtained from the unthinned plants than from the plants spaced at 12 inches. An examination was therefore made of 10-foot sections of the inside rows of each 4-row block in the experiment, and the 4-lock and 5-lock bolls were counted. A small but significant difference was found in the percentages of 4-lock bolls in the two spacings, there being 38.52 ± 1.17 percent in the unthinned plants and 32.66 ± 1.16 percent in the 12-inch-spaced plants, or a difference of 5.86 ± 1.65 percent. A similar tendency appeared in the comparison of one and two plants per foot. Though the percentage differences were small, they indicated that the spacing of the plants had an effect on the proportion of 4-lock bolls produced.

EXPERIMENTS IN 1927

In 1927 somewhat more comprehensive data were obtained on the effect of spacing on the number of locks in the boll; the effect of the 2-plant and blocked-out spacings was studied also. In the 2-plant test, three 4-row blocks of this spacing alternated with three 4-row blocks in which the spacing was 1 plant to 12 inches. Similarly, in the blocked-out test, three 4-row blocks of blocked-out plants alternated with 3 blocks of the 12-inch spacing, but in the unthinned test there were only two 4-row blocks of each spacing. The data were obtained from 300 bolls on scattered small groups of contiguous plants selected at random in the inner rows of each 4-row block. In both the 2-plant and blocked-out tests, therefore, 900 bolls were examined for each method of spacing; in the unthinned test only 600 bolls were examined for each spacing.

The number of 4-lock and 5-lock bolls and the percentage of 4-lock bolls by separate blocks and by totals, with the average number of plants per foot are shown in table 1.

No difference was found in the proportion of 4-lock bolls in the test of one and two plants per foot, both spacings showing 21.88 ± 0.93 percent. The least difference in the average number of plants per foot occurred in this test, the 1-plant spacing averaging 1.15 plants per foot and the 2-plant spacing averaging 1.62. There was apparently no crowding effect in this test.

TABLE 1.—*Number of 4-lock and 5-lock bolls, percentage of 4-lock bolls, and average number of plants per row foot in spacing experiments, 1927*

Test no., block no., and treatment	Average number of plants per row foot	4-lock bolls	5-lock bolls	4-lock bolls
		Number	*Number*	*Percent*
Test 1:				
Block 1 (1 plant to 12 inches)	1.03	65	235	21.67±1.60
Block 2 (blocked out)	2.62	95	205	31.67±1.81
Block 3 (1 plant to 12 inches)	.99	78	222	26.00±1.71
Block 4 (blocked out)	2.56	122	178	40.67±1.91
Block 5 (1 plant to 12 inches)	.98	73	227	24.33±1.67
Block 6 (blocked out)	2.40	91	209	30.33±1.79
Total (1 plant to 12 inches)	1.00	216	684	24.00± .96
Total (blocked out)	2.53	308	592	34.22±1.07
Test 2:				
Block 1 (1 plant to 12 inches)	1.18	73	227	24.33±1.67
Block 2 (2 plants to 12 inches)	1.68	62	238	20.67±1.58
Block 3 (1 plant to 12 inches)	1.13	62	238	20.67±1.58
Block 4 (2 plants to 12 inches)	1.61	67	233	22.33±1.62
Block 5 (1 plant to 12 inches)	1.13	62	238	20.67±1.58
Block 6 (2 plants to 12 inches)	1.58	68	232	22.67±1.63
Total (1 plant to 12 inches)	1.15	197	703	21.88± .93
Total (2 plants to 12 inches)	1.62	197	703	21.88± .93
Test 3:				
Block 1 (1 plant to 12 inches)	1.43	80	220	26.67±1.72
Block 2 (unthinned)	5.85	138	162	46.00±1.94
Block 3 (1 plant to 12 inches)	1.44	66	234	22.00±1.61
Block 4 (unthinned)	4.71	109	191	36.33±1.87
Total (1 plant to 12 inches)	1.44	146	454	24.33±1.18
Total (unthinned)	5.28	247	353	41.17±1.31

In the blocked-out test, the 12-inch-spaced plants produced 24 ± 0.96 percent of 4-lock bolls and the blocked-out plants 34.22 ± 1.07 percent, a difference of 10.22 ± 1.44 per cent. The average number of plants per foot was 1.0 and 2.53 for the wider and closer spacings, respectively. The effect of closer spacing was apparent here.

In the unthinned plants there was an average of 41.17 ± 1.31 percent of 4-lock bolls, while the compared blocks of 12-inch-spaced plants produced 24.33 ± 1.18 percent, a difference of 16.84 ± 1.76 percent. The average number of plants per foot in the unthinned blocks was 5.28 and in the 12-inch blocks 1.44. The effect of crowding on the production of 4-lock bolls appeared very definite in this test.

EXPERIMENTS IN 1928

The study of spacing effect was continued in the 1928 tests, the same method for obtaining the data being used as in the previous season. The data are presented in table 2.

The greatest differences in the numbers of 4-lock bolls produced were again found where the greatest differences in plant spacing existed. In the unthinned blocks the plants averaged 5.60 per foot, while in the 12-inch blocks with which they were compared there were but 1.01 plants per foot. A greater difference was found between the percentages of 4-lock bolls in these spacings than in 1927, the unthinned plants having 39.11 ± 1.10 percent and the 12-inch-spaced plants 18.22 ± 0.87 percent, a difference of 20.89 ± 1.40 percent. The relation of the percentages in the separate blocks to the number of plants per foot, however, was not as uniform as in 1927. In test 3 there was

a difference of 23 per cent between blocks 2 and 4, although the difference in the number of plants per foot was relatively small. On the other hand, block 6, where the number of plants was substantially greater than in either block 2 or block 4, had fewer 4-lock bolls than the former, the difference being approximately 20 percent, and but a few more 4-lock bolls than the latter, the difference in this case being but a little over 2 percent.

TABLE 2.—*Number of 4-lock and 5-lock bolls, percentage of 4-lock bolls, and average number of plants per row foot in spacing experiments, 1928*

Test no., block no., and treatment	Average number of plants per row foot	4-lock bolls	5-lock bolls	4-lock bolls
Test 1:		*Number*	*Number*	*Per cent*
Block 1 (1 plant to 12 inches)	0.97	65	235	21.67±1.60
Block 2 (blocked out)	3.46	87	213	29.00±1.76
Block 3 (1 plant to 12 inches)	1.09	37	263	12.33±1.28
Block 4 (blocked out)	3.13	100	200	33.33±1.84
Block 5 (1 plant to 12 inches)	1.05	45	255	15.00±1.39
Block 6 (blocked out)	3.35	86	214	28.67±1.86
Total (1 plant to 12 inches)	1.04	147	753	16.33± .83
Total (blocked out)	3.31	273	627	30.33±1.03
Test 2:				
Block 1 (1 plant to 12 inches)	1.08	52	248	17.33±1.47
Block 2 (2 plants to 12 inches)	1.84	69	231	23.00±1.64
Block 3 (1 plant to 12 inches)	1.09	37	263	12.33±1.28
Block 4 (2 plants to 12 inches)	1.90	64	236	21.33±1.60
Block 5 (1 plant to 12 inches)	1.01	64	236	21.33±1.60
Block 6 (2 plants to 12 inches)	1.83	71	229	23.67±1.65
Total (1 plant to 12 inches)	1.06	153	747	17.00± .84
Total (2 plants to 12 inches)	1.86	204	696	22.67± .94
Test 3:				
Block 1 (1 plant to 12 inches)	.99	44	256	14.67±1.38
Block 2 (unthinned)	5.52	161	139	53.67±1.94
Block 3 (1 plant to 12 inches)	1.01	56	244	18.67±1.52
Block 4 (unthinned)	5.20	92	208	30.67±1.80
Block 5 (1 plant to 12 inches)	1.04	64	236	21.33±1.60
Block 6 (unthinned)	6.09	99	201	33.00±1.83
Total (1 plant to 12 inches)	1.01	164	736	18.22± .87
Total (unthinned)	5.60	352	548	39.11±1.10

The smallest differences in the number of plants per foot occurred in the test of one and two plants to 12 inches, and correspondingly smaller differences in the 4-lock percentages were found. These differences, however, were greater than they had been in 1927, and the individual percentages showed wider variations. Of the total number of bolls examined in the 1-plant spacings, 17 ± 0.84 percent were 4 lock, while in the 2-plant spacings 22.67 ± 0.94 percent were 4 lock, the difference being 5.67 ± 1.26 percent, which was probably significant.

A fairly uniform response of the 4-lock percentage to the number of plants per foot was noted in the blocked-out test. The average number of plants per foot was 1.04 for the 1-plant spacings and 3.31 for the blocked out, and the percentages of 4-lock bolls were 16.33 ± 0.83 in the former spacing and 30.33 ± 1.03 in the latter, the difference being 14 ± 1.32 percent. The 4-lock percentages in both spacings were lower than in 1927, although the average number of plants per foot was higher.

Two other spacing tests were included in the 1928 work, these being the comparison of the standard 12-inch spacing with 18-inch and 24-

inch spacings, respectively. Boll counts were made in these tests, as in the others, but no significant differences in the percentages of 4-lock bolls were found.

EXPERIMENTS IN 1929

Further data on the effect of plant spacing were obtained in 1929 from the spacing tests, which consisted of comparisons of the standard 12-inch spacing with blocked-out, unthinned, 18-inch, and 24-inch spacings. The two latter tests again failed, as in the preceding year, to show any significant differences in percentages of 4-lock bolls. In the blocked-out and unthinned tests, however, effects similar to those of the two previous years were observed, with slightly greater differences between the 4-lock percentages in the wide and close spacings than in either 1927 or 1928. The data are shown in table 3.

TABLE 3.—*Number of 4-lock and 5-lock bolls, percentage of 4-lock bolls, and average number of plants per row foot in spacing experiments, 1929*

Test no., block no., and treatment	Average number of plants per row foot	4-lock bolls	5-lock bolls	4-lock bolls
Test 1:		*Number*	*Number*	*Percent*
Block 1 (1 plant to 12 inches)	0.97	69	231	23.00±1.64
Block 2 (blocked out)	3.34	129	171	43.00±1.93
Block 3 (1 plant to 12 inches)	.97	55	245	18.33±1.51
Block 4 (blocked out)	3.54	117	183	39.00±1.90
Block 5 (1 plant to 12 inches)	.98	75	225	25.00±1.69
Block 6 (blocked out)	3.03	92	208	30.67±1.80
Total (1 plant to 12 inches)	.97	199	701	22.11± .93
Total (blocked out)	3.30	338	562	37.55±1.09
Test 2:				
Block 1 (1 plant to 12 inches)	.94	75	225	25.00±1.69
Block 2 (unthinned)	5.64	167	133	55.67±1.93
Block 3 (1 plant to 12 inches)	.92	62	238	20.67±1.58
Block 4 (unthinned)	5.80	159	141	53.00±1.94
Block 5 (1 plant to 12 inches)	.94	59	241	19.67±1.55
Block 6 (unthinned)	6.37	135	165	45.00±1.94
Total (1 plant to 12 inches)	.93	196	704	21.77± .93
Total (unthinned)	5.94	461	439	51.22±1.12

In the test in which the 12-inch and blocked-out spacings were compared, the plants averaged 0.97 per foot in the former spacing and 3.30 per foot in the latter, and the 4-lock percentages were 22.11 ± 0.93 and 37.55 ± 1.09, respectively, with a difference of 15.44 ± 1.43 percent. In the unthinned blocks the plants averaged 5.94 per foot, with 51.22 ± 1.12 percent of 4-lock bolls, while in the compared 12-inch blocks they averaged 0.93 per foot, with 21.77 ± 0.93 percent of 4-lock bolls, a difference of 29.45 ± 1.46 percent. In the 12-inch spacings the 4-lock percentages were slightly higher than in 1928 and not quite so high as in 1927, whereas in the closer spaced blocks they were higher than in either of the two previous years, especially in the unthinned plants. It will be seen from table 3 that again the number of plants per foot had a marked effect on the 4-lock percentages, failing in but one instance to show a positive reaction. In the unthinned plants in block 6 there were fewer 4-lock bolls than in either of the other similarly spaced blocks, the percentage being 45 ± 1.94, with differences of 10.67 ± 2.74 and 8 ± 2.74 percent between this block and blocks 2 and 4, respectively, although the average number of plants was greater than in either of the latter.

EFFECT OF IRRIGATION AND SPACING

EXPERIMENT IN 1928

In addition to the regular spacing tests in 1928, a separate experiment was carried on to furnish additional data. Since the evidence thus far had clearly indicated that the crowding of the plants in the row had a material effect upon the proportion of 4-lock bolls produced, the question arose as to what extent other factors might be involved. The amount of moisture in the soil being a known cause of accelerated or retarded growth and being to some extent controllable under irrigation, this factor was included with the spacing of the plants.

In this experiment one of the plots at the field station was laid off into four equal parts or sections, each section being wide enough for 10 rows of plants, spaced 3½ feet apart, or sufficient for two 4-row blocks with "guard" rows on the outer sides. In order to accentuate the difference in the spacings, an 18-inch instead of a 12-inch spacing was used in comparison with unthinned plants. In each section, five rows were thinned to the 18-inch spacing and five rows were left unthinned. Two of the sections, nos. 2 and 4, were given the normal number of irrigations during the season; the requirements being judged by the general growth and behavior of the plants. The other two sections, nos. 1 and 3, were subjected to a very restricted irrigation program, the plants being made to suffer from lack of moisture during periods of high temperature during the summer. By this arrangement the plants were grown under two widely contrasted methods of spacing and each spacing subjected to two conditions of soil moisture.

Under the normal-irrigation program, sections 2 and 4 were given six irrigations, on May 11, June 14, July 11, August 1 and 15, and September 12, respectively. Sections 1 and 3 received two less irrigations than sections 2 and 4, water being applied on May 11, July 11, August 8, and September 12.

A slight difference in the size of the plants was first noted toward the middle of July, but little effect of water stress as evidenced by wilting was apparent at that time. The extremely dry condition desired in sections 1 and 3 was interfered with to some extent by the summer rains. Several light showers occurred during May, but these were insufficient to materially affect the soil moisture, although they increased the atmospheric humidity. No rain occurred during June, but on July 19 and 20, 0.88 inch of rain fell. During August there were rains of 0.29 inch on the 1st, 0.79 inch on the 17th, and 0.34 inch on the 30th, and several light showers between. These rains relieved the plants to some extent from the extremely dry conditions that would otherwise have prevailed, and mitigated the severity of the treatment of sections 1 and 3. Some early-morning wilting of the plants in these sections, however, was noted during the first part of August, with more severe wilting during the afternoons. This was in contrast with the unwilted condition of the plants in sections 2 and 4.

Table 4 shows the data covering the results of this experiment, which were obtained in the same manner as in the regular spacing tests, 300 bolls on adjacent plants in the inside rows of each block being examined.

Effects similar to those in the regular spacing tests were encountered in this experiment, but they appeared to be influenced by irrigation.

The percentage of 4-lock bolls in the unthinned plants was higher in each case than in 18-inch-spaced plants, but the differences were greater in the sections under the light irrigation treatment than they were under normal irrigation. In the combined unthinned sections under light irrigation there was approximately 46 percent of 4-lock bolls, while in the combined 18-inch sections the percentage was approximately 20, a difference of about 26 percent. In the combined sections under normal irrigation the percentages were approximately 30 for the unthinned and 20 for the 18-inch, with a difference of a little over 10 percent. The difference in 4-lock percentage in the combined sections was thus about two and one-half times as great under light irrigation as under normal irrigation. Differences between combined sections due to irrigation treatment were about 16 percent in the unthinned plants and less than 1 percent in the 18-inch-spaced plants. From this experiment it was therefore apparent that the close spacing of the plants was a more important factor in the production of higher percentages of 4-lock bolls than the reduced amount of irrigation water, and also that the reduced moisture affected the unthinned plants to a much greater extent than the wide-spaced plants.

TABLE 4.—*Number of 4-lock and 5-lock bolls, percentage of 4-lock bolls, and average number of plants per row foot, with differences due to irrigation treatment and spacing in irrigation-spacing experiment, 1928*

Section no. and treatment	1 plant to 18 inches				Unthinned				Difference in percentage of 4-lock bolls due to spacing
	Average number of plants per row foot	4-lock bolls	5-lock bolls	4-lock bolls	Average number of plants per row foot	4-lock bolls	5-lock bolls	4-lock bolls	
		Number	*Number*	*Percent*		*Number*	*Number*	*Percent*	
Section 1 (light irrigation)	0.68	54	246	18.00±1.50	4.05	133	167	44.33±1.93	26.33±2.44
Section 2 (normal irrigation)	.69	64	236	21.33±1.59	5.40	83	217	27.67±1.72	6.34±2.34
Section 3 (light irrigation)	.69	68	232	22.67±1.63	5.69	142	158	47.33±1.94	24.66±2.53
Section 4 (normal irrigation)	.70	54	246	18.00±1.50	5.11	98	202	32.67±1.83	14.67±2.37
Sections 1 and 3 (light) combined	.69	122	478	20.33±1.11	4.87	275	325	45.83±1.37	25.50±1.76
Sections 2 and 4 (normal) combined	.70	118	482	19.67±1.09	5.26	181	419	30.17±1.26	10.50±1.66
Difference due to irrigation treatment				.66±1.56				15.66±1.86	

EXPERIMENT IN 1929

During the season of 1929 further studies on the effect of irrigation in connection with spacing were carried on. The experiment was arranged and the plantings made as in 1928. In order, however, to accentuate the stress conditions over those of the previous season, even less irrigation water was applied to sections 1 and 3. The plants treated in this manner are referred to as "stressed," in contrast to those subjected the previous year to "light irrigation."

Under the normal method of irrigation the plants in sections 2 and 4 received five irrigations, on June 11, July 9 and 30, August 20, and September 17, respectively. Sections 1 and 3 were irrigated only twice, on June 11 and on August 6. Greater severity of water stress

was induced in these two sections than during the previous season, and there was a more notable difference in the growth and behavior of the plants. A few showers occurred during May, with a precipitation of 0.76 inch of rain on the 21st. June was without rainfall. During July 1.52 inches of rain fell on the 9th and 10th, and 0.45 inch on the 19th, with an occasional light shower toward the end of the month. On August 4, 0.24 inch of rain fell; on August 10 to 12, 1.13 inches; and on August 28 and 29, 0.69 inch. There were showers on August 8, 9, and 21, but they had little effect on the plants. During September 0.08 inch of rain fell on the 5th, 0.61 inch on the 22d, and 0.13 inch on the 24th. A greater amount of rain fell during May, July, and August than in the same months in 1928, but owing to the fewer irrigations, the plants in sections 1 and 3 were subjected to more stringent conditions. For a considerable period before the irrigation of August 6, the plants in these two sections had given indications of water shortage. They were badly wilted during most of the daylight hours, and the rate of growth and flowering was much less than in the normally irrigated sections. After the irrigation of August 6 they took on a more normal appearance, which was maintained for several days, until stress was again evidenced. On August 9 the average height of the plants in both the 18-inch and unthinned spacings of these sections was approximately 24 inches. Plants in the normally irrigated sections were from 36 to 44 inches high, the unthinned plants being a little taller than the wide-spaced plants.

A different procedure for obtaining the data was followed during the 1929 season. Since it is possible that the ultimate proportions of 4-lock and 5-lock bolls may be affected by differential shedding, as shown by Beckett and Hubbard (*2*), the effects of growth conditions on the number of locks or carpels may be shown more definitely in the flowers than in the bolls. It is also possible to obtain a much larger array of data from the flowers than from the mature bolls, since there is considerable shedding of squares and young bolls after flowering. Beginning on July 25, therefore, when flowering had become general, and continuing until September 27, 25 flowers in each spacing of each section were inspected daily, the number of flowers with four or five carpels being determined by the stigma lobes. By this method it was not only possible to obtain more comprehensive data, but also to follow the fluctuations in the rate of production of potential 4-lock and 5-lock bolls during the season. The subsequent tables and figures pertaining to this phase of the 1929 work, therefore, refer to the number of carpels in the flowers instead of locks in the bolls, as in the tables covering the previous work.

During the season 9,605 flowers were examined, of which 5,386 occurred in the normally irrigated sections and 4,219 in the stressed sections. When the restricted conditions of growth that prevailed at certain periods and toward the end of the season made it impossible to obtain a daily count of 25 flowers, all the flowers on the inner rows of the blocks, except those on end plants, were examined. The total number of flowers under stressed conditions comprised 2,147 from section 1 and 2,072 from section 3. Sections 2 and 4, under normal irrigation, had 2,677 and 2,709 flowers, respectively. The similarity in the number of flowers in sections 1 and 3 and sections 2 and 4 indicates that the two methods of irrigation had like effects in each of the two sections in which they were applied and that they are therefore comparable.

The number of flowers with 4 carpels and 5 carpels and the percentage of flowers with 4 carpels in the various sections and blocks are shown in table 5. In order to simplify the table, the time covered by the experiment was divided into 5-day periods, and the data are shown for such periods.

On the basis of 5-day periods it was possible to make 52 comparisons between the 18-inch and the unthinned spacings in all four sections of the experiment, or 26 comparisons in each irrigation treatment. These comparisons show that in all cases under normal irrigation, and in all but one case under the stress treatment, the unthinned plants had higher percentages of flowers with 4 carpels than the 18-inch-spaced plants. Since there were 2 days in August and 5 days in September when observations were not made, only 232 out of a possible 260 comparisons were made on the basis of daily flower counts; of these, 201 showed higher percentages of fours in the unthinned plants, 20 showed higher percentages of fours in the 18-inch-spaced plants, and 11 showed equal percentages in both spacings. Of the 116 comparisons under the stress treatment, 99 showed higher percentages of flowers with 4 carpels in the unthinned plants, 10 in the 18-inch spacing, and 7 were equal. Of comparisons in the normally irrigated sections, 102 showed higher 4-carpel percentages in the unthinned plants, 10 in the 18-inch spacing, and 4 were equal.

In the day-to-day examination of the flowers, 174 comparisons of the effects of the two irrigation treatments were afforded in each of the two spacings by comparing similar spacings in the sections which adjoined; that is, section 1 with section 2, section 2 with section 3, etc. In the 18-inch spacing, 107 of the 174 daily comparisons of the percentages of flowers with 4 carpels showed higher percentages under stress conditions, 59 under normal irrigation, and 8 were equal. In the unthinned blocks, out of the 174 daily comparisons, 121 showed higher percentages under the stress treatment, 42 under normal irrigation, and 11 were equal.

The differences between the percentages of flowers with four carpels as affected by spacing were materially greater, on the whole, than as affected by the reduction of moisture in the soil.

The data are also shown in four charts (figs. 1, 2, 3, and 4), two of which compare the figures on the basis of spacing and two on the basis of irrigation treatment, arranged by 5-day periods.

From figure 1 it will be noted that from the beginning of the daily examinations the percentages of flowers having four carpels were higher in the unthinned than in the 18-inch-spaced plants, and remained so throughout almost the entire period of observation. The percentages for the two sections of unthinned plants mounted rapidly from the end of July to the middle of August, and remained between 75 and 95 until the latter part of September. The percentages for the 18-inch spacings had a more gradual rise throughout the season until late September when, like those for the unthinned plants, they dropped very sharply The greatest diversity between spacings in the rate of 4-carpel flower production occurred from about August 14 until about September 2, during which time the average difference was nearly 46 percent. The curves representing each of the two blocks of the same spacing in the different sections remained fairly constant in relation to each other, indicating that similar conditions prevailed in the companion blocks.

TABLE 5.—*Number of flowers with 4 carpels and 5 carpels and percentage of flowers with 4 carpels in irrigation-spacing experiment, by 5-day periods, July 25 to Sept. 27, 1929*

5-day period	Section 1 (stress conditions)						Section 2 (normal irrigation)					
	1 plant to 18 inches			Unthinned			1 plant to 18 inches			Unthinned		
	Flowers with—		Flowers with 4 carpels	Flowers with—		Flowers with 4 carpels	Flowers with—		Flowers with 4 carpels	Flowers with—		Flowers with 4 carpels
	4 carpels	5 carpels		4 carpels	5 carpels		4 carpels	5 carpels		4 carpels	5 carpels	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
July 25 to 29	17	107	13.71±2.08	23	101	18.55±2.35	18	106	14.52±2.13	35	90	28.00±2.71
July 30 to Aug. 3	19	105	15.32±2.18	28	97	22.40±2.52	13	110	10.57±1.87	30	95	24.00±2.58
Aug. 4 to 8	19	81	19.00±2.65	52	48	52.00±3.37	23	77	23.00±2.84	44	56	44.00±3.35
Aug. 9 to 13	46	79	36.80±2.91	74	25	74.75±2.95	24	101	19.20±2.38	90	35	72.00±2.71
Aug. 14 to 18	44	79	35.77±2.92	58	10	85.29±2.89	28	97	22.40±2.52	96	28	77.42±2.53
Aug. 19 to 23	45	67	40.18±3.12	22	7	75.86±5.36	41	84	32.80±2.83	73	52	58.40±2.97
Aug. 24 to 28	18	25	41.86±5.07	15	3	83.33±5.92	30	70	30.00±3.09	55	45	55.00±3.37
Aug. 29 to Sept. 2	8	5	61.54±9.09	11	1	91.67±5.38	35	90	28.00±2.71	79	41	65.83±2.92
Sept. 3 to 7	51	29	63.75±3.63	50	10	83.33±3.24	34	91	27.20±2.69	80	45	64.00±2.90
Sept. 8 to 12	58	41	58.59±3.34	91	9	91.00±1.93	45	43	51.14±3.59	52	26	66.67±3.60
Sept. 13 to 17	66	31	68.04±3.19	94	6	94.00±1.60	52	13	80.00±3.35	70	12	85.37±2.63
Sept. 18 to 22	45	9	83.33±3.42	70	5	93.33±1.94	25	13	65.79±5.19	56	7	88.89±2.67
Sept. 23 to 27	22	21	51.16±5.14	48	52	48.00±3.37	17	17	50.00±5.79	64	24	72.73±3.20
Total	458	679	40.28± .98	636	374	62.97±1.02	385	912	29.68± .86	824	556	59.71± .89

TABLE 5.—*Number of flowers with 4 carpels and 5 carpels and percentage of flowers with 4 carpels in irrigation-spacing experiment, by 5-day periods, July 25 to Sept. 27, 1929*—Continued

5-day period	Section 3 (stress conditions)						Section 4 (normal irrigation)					
	1 plant to 18 inches			Unthinned			1 plant to 18 inches			Unthinned		
	Flowers with—		Flowers with 4 carpels	Flowers with—		Flowers with 4 carpels	Flowers with—		Flowers with 4 carpels	Flowers with—		Flowers with 4 carpels
	4 carpels	5 carpels		4 carpels	5 carpels		4 carpels	5 carpels		4 carpels	5 carpels	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
July 25 to 29	13	112	10.40±1.84	28	96	22.58±2.53	19	106	15.20±2.17	28	96	22.58±2.53
July 30 to Aug. 3	12	113	9.60±1.78	36	88	29.03±2.75	22	103	17.60±2.30	31	94	24.80±2.61
Aug. 4 to 8	23	77	23.00±2.84	44	56	44.00±3.35	14	86	14.00±2.34	17	83	17.00±2.53
Aug. 9 to 13	24	101	19.20±2.38	39	13	75.00±4.05	20	105	16.00±2.21	76	49	60.80±2.95
Aug. 14 to 18	29	96	23.20±2.55	23	2	92.00±3.66	19	106	15.20±2.17	114	11	91.20±1.71
Aug. 19 to 23	40	67	37.38±3.16	14	1	93.33±4.35	19	106	15.20±2.17	86	39	68.80±2.79
Aug. 24 to 28	12	21	36.36±5.65	10	1	90.91±5.84	24	76	24.00±2.88	46	54	46.00±3.36
Aug. 29 to Sept. 2	17	12	58.62±6.16	9	1	90.00±6.40	31	94	24.80±2.61	60	65	48.00±3.01
Sept. 3 to 7	32	39	45.07±3.98	66	15	81.48±2.91	33	92	26.40±2.66	96	29	76.80±2.55
Sept. 8 to 12	50	50	50.00±3.37	84	16	84.00±2.47	54	32	62.79±3.52	72	21	77.42±2.93
Sept. 13 to 17	70	27	72.16±3.07	94	6	94.00±1.60	53	17	75.71±3.46	70	8	89.74±2.32
Sept. 18 to 22	51	8	86.44±3.01	68	7	90.67±2.26	27	10	72.97±4.93	64	10	86.49±2.68
Sept. 23 to 27	31	28	52.54±4.39	55	45	55.00±3.36	17	22	43.59±5.35	57	26	68.67±3.43
Total	404	751	34.98± .95	570	347	62.16±1.08	352	955	26.93± .83	817	585	58.27± .89

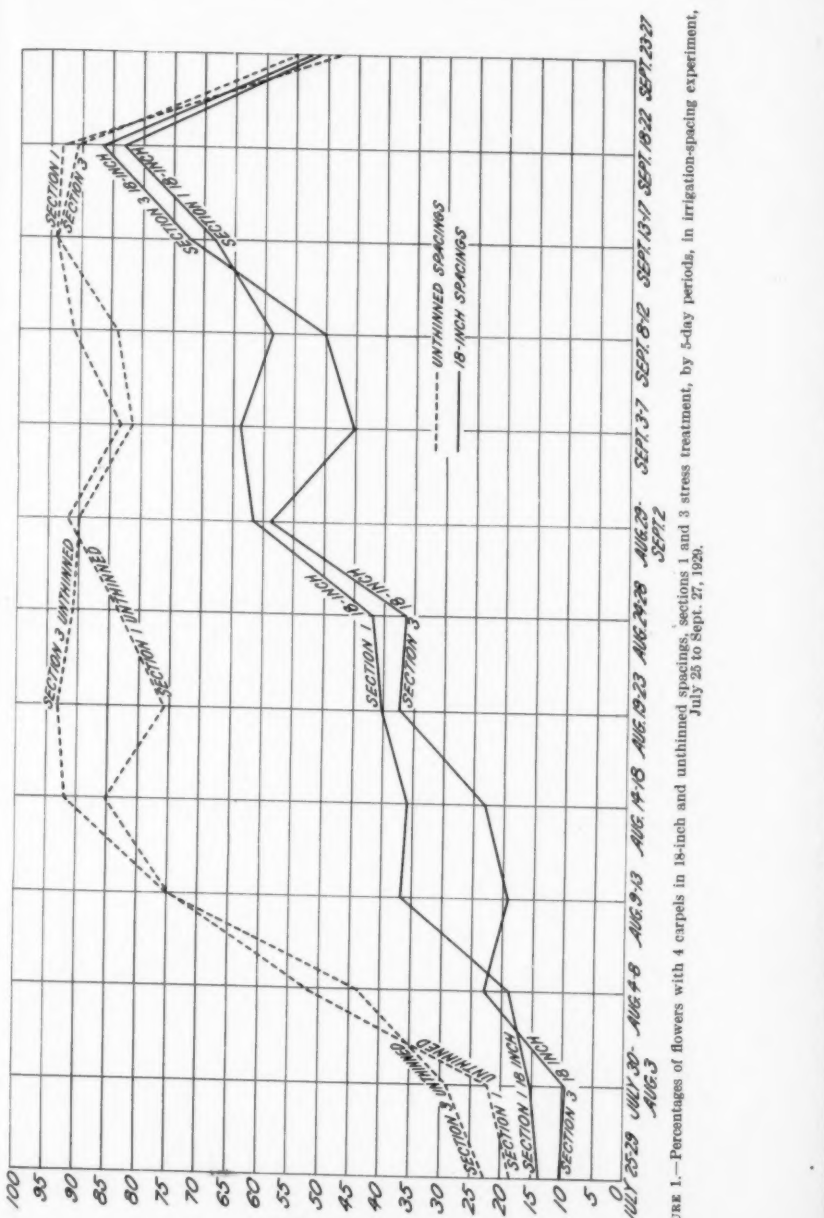

FIGURE 1.—Percentages of flowers with 4 carpels in 18-inch and unthinned spacings, sections 1 and 3 stress treatment, by 5-day periods, in irrigation-spacing experiment, July 25 to Sept. 27, 1928.

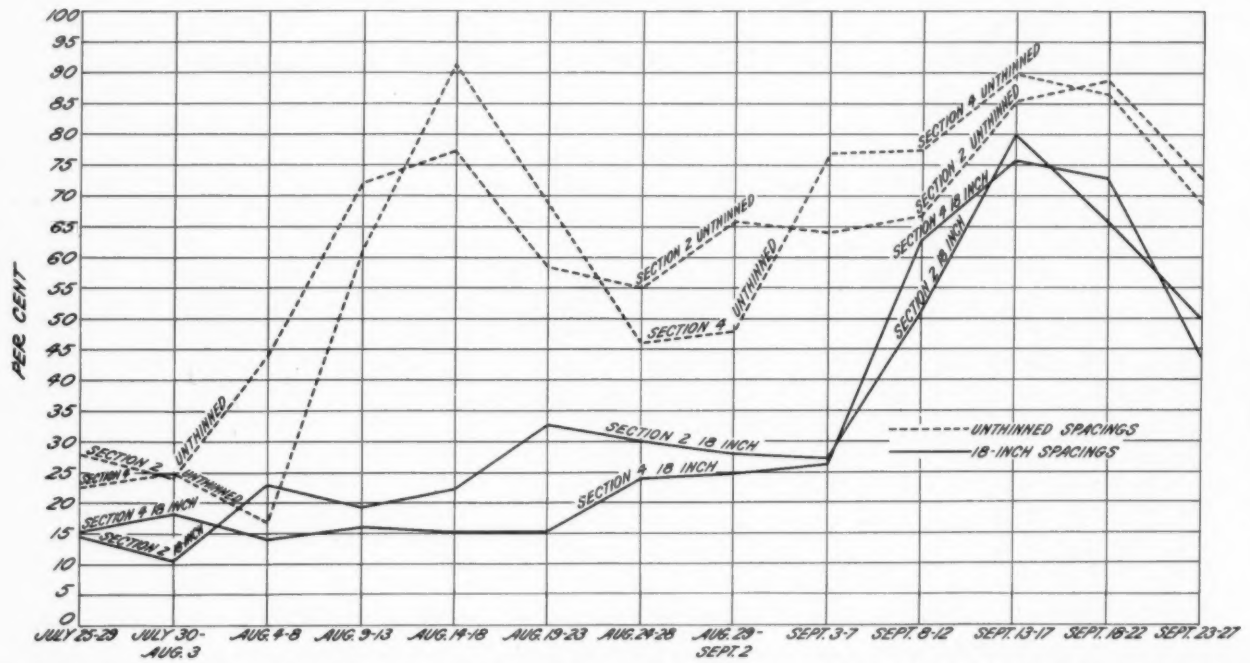

FIGURE 2.—Percentages of flowers with 4 carpels in 18-inch and unthinned spacings, sections 2 and 4, normal irrigation, by 5-day periods, in irrigation-spacing experiment, July 25 to Sept. 27, 1929.

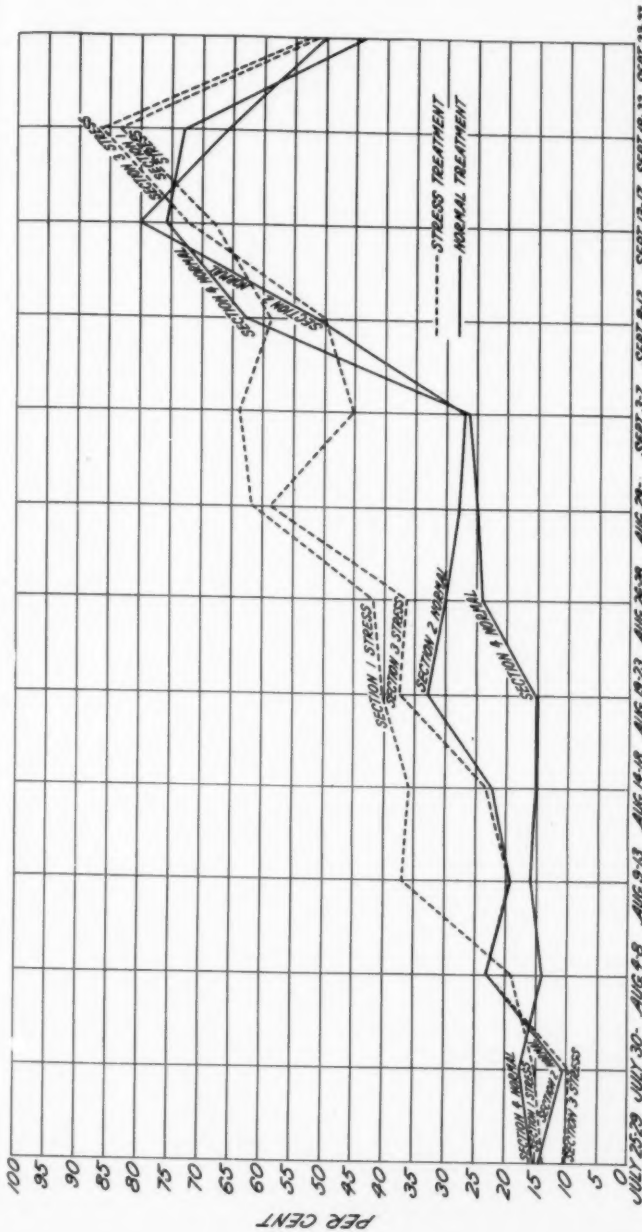

FIGURE 3.—Percentages of flowers with 4 carpels under normal irrigation and stress treatment in 18-inch spaced plants, sections 1, 2, 3, and 4, by 5-day periods, in irrigation-spacing experiment, July 25 to Sept. 27, 1929.

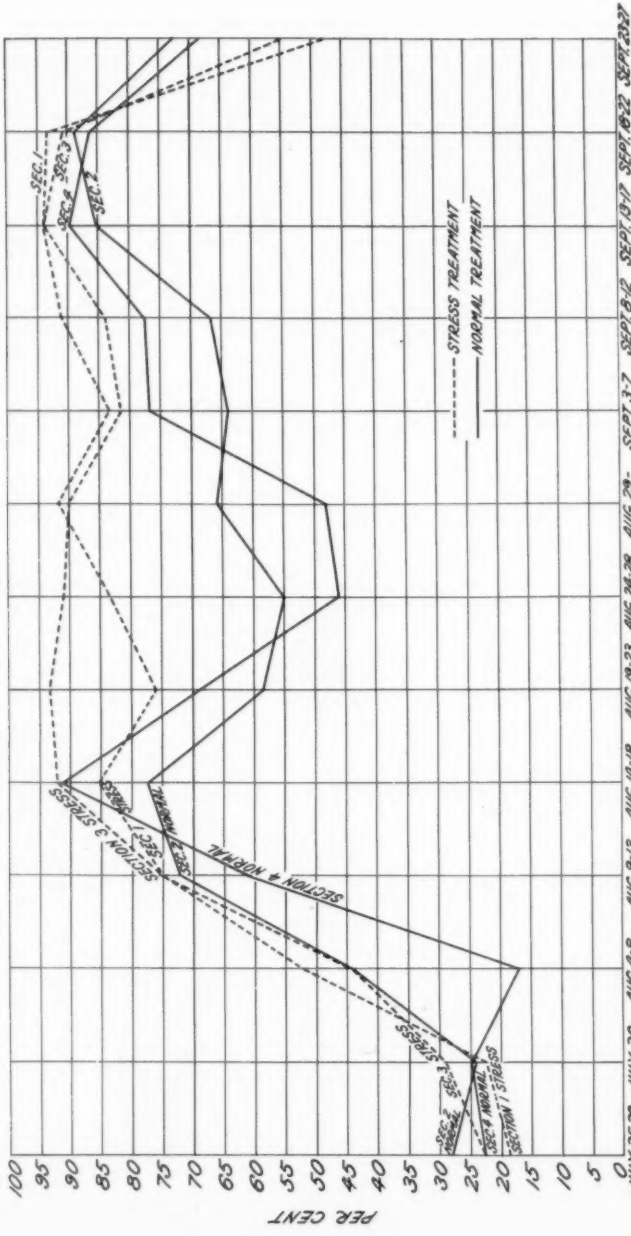

FIGURE 4.—Percentages of flowers with 4 carpels under normal irrigation and stress treatment in unthinned plants, sections 1, 2, 3, and 4, by 5-day periods, in irrigation-spacing experiment, July 25 to Sept. 27, 1928.

During the entire season the percentages of flowers with four carpels were not so high for either spacing under normal irrigation (fig. 2) as under stress conditions (fig. 1). The percentage of fours in the 18-inch spacings remained fairly low from July 25 to about September 7, when it averaged approximately 27. During the ensuing 10-day period there were sharp rises in the percentages in both sections until the average was approximately 78, a difference of over 50 percent. From this peak of 4-carpel flower production, about September 17, the percentage dropped to approximately 47 on September 27, a difference of over 30 percent. In the unthinned plants the 4-carpel percentages rose very rapidly in the early part of the season, increasing from approximately 24 percent in the period from July 30 to August 3 to an average of over 84 percent by August 18, a difference of over 60 percent. The percentages then declined sharply during the last two 5-day periods. The reasons for sudden rises and falls in the 4-carpel percentages are undetermined, but are probably associated with cultural conditions previously encountered by the plants.

In figure 3 the data have been arranged to show the effect of the contrasting moisture conditions upon the production of flowers with four carpels in the 18-inch-spaced plants. The curves indicate that during the 15 days after observations were begun there were only slight differences in the percentages in the two treatments. After August 8, however, the stressed plants began to produce more flowers with four carpels, and this was continued for over a month. The marked rises in the percentages in the stressed sections after August 28 were not followed by a rise in the percentages in the normally irrigated sections, where for 10 days longer the percentages remained low. A very sharp rise then occurred in the percentages in the normally irrigated blocks, until for the only time during the season they exceeded those in both the stressed sections. This condition lasted for but a few days, when the percentages under the normal treatment again fell below those under the stress treatment.

Figure 4 shows the percentages of 4-carpel flowers in the unthinned plants under the two irrigation treatments in the same manner that figure 3 shows those in the 18-inch spacings. By comparing figures 3 and 4, it will be seen that the percentages of flowers with 4 carpels were higher in the unthinned plants throughout most of the season. As between irrigation treatments (fig. 4), the percentages in the stressed sections were somewhat higher than in the normally irrigated sections, with very pronounced differences occurring during the middle and latter part of August and early September. Toward the end of the season the percentages became fairly uniform for about 10 days and then dropped sharply, when the stressed sections showed greater reductions than did the normally irrigated sections.

During the earlier part of the season the percentages of flowers with 4 carpels in both the normally irrigated and stressed sections increased much more rapidly in the unthinned plants than in the 18-inch spacings. During the middle of August, the proportion of fours in the unthinned plants was very high, particularly in the stressed sections. The 18-inch spacings, on the whole, showed a more gradual rise in 4-carpel percentages as the season progressed, which may be considered a normal occurrence. From September 23 to 27 a marked drop occurred in the percentages in both spacings and both irrigation treatments. This sharp reduction in the proportion of 4-carpel

flowers in all the plants, regardless of spacing or irrigation treatment, apparently resulted from the same cause, which was probably physiological.

A comparison of figures 1 and 2 with figures 3 and 4 shows that during most of the season the proportion of 4-carpel flowers produced depended upon spacing to a considerably greater extent than upon irrigation. In other words, in the production of flowers having four carpels, the crowding of the plants was a more important factor than the stress conditions resulting from reduced soil moisture.

Table 6 shows the average number of plants per foot and the average percentages of flowers with four carpels under the different spacing and irrigation treatments for the entire season of 1929. The differences in the percentages due to irrigation were relatively small, only 3.59 ± 0.97 in the unthinned plants and 9.31 ± 0.91 in the 18-inch spaced plants, the stressed sections having the higher percentages in both cases. However, it is probable that the small percentage difference in the unthinned plants was partly due to the fact that the normally irrigated sections were somewhat more crowded, as they averaged one plant more to the foot than the stressed sections. The effect of stress conditions appears to have been greater in the wide-spaced than in the crowded plants, since the difference in the 4-carpel percentage was nearly three times as large in the former as in the latter, but it is likely that this difference would have been less had the unthinned plants under stress treatment been as closely spaced as those under normal irrigation. The differences resulting from irrigation treatment were much smaller than those due to plant spacing. There was a difference of 24.97 ± 1.00 between the 18-inch and unthinned plants in the percentage of flowers with 4 carpels under the stress treatment, and under normal irrigation the difference was 30.69 ± 0.87, the unthinned plants having the higher percentages.

TABLE 6.—*Percentage of flowers with 4 carpels and average number of plants per row foot, with differences due to irrigation treatment and spacing, covering the entire season, in irrigation-spacing experiment, 1929*

Section no. and treatment	18-inch spacing		Unthinned		Difference due to spacing
	Average number of plants per row foot	Percentage of flowers with 4 carpels	Average number of plants per row foot	Percentage of flowers with 4 carpels	
Sections 1 and 3 (stress) combined	0.66	37.61±0.68	4.78	62.58±0.74	24.97±1.00
Sections 2 and 4 (normal) combined	.67	28.30± .60	5.78	58.99± .63	30.69± .87
Difference due to irrigation treatment		9.31± .91		3.59± .97	

A comparison of table 6 and table 4 shows that the percentages of flowers with 4 carpels in 1929 was greater than the 4-lock boll percentages in 1928, although the averages of the number of plants per foot were but slightly different in the two years. The difference in the percentages was most apparent in the normally irrigated sections, especially among the unthinned plants. As already mentioned, similar differences were encountered in the regular spacing tests for these two years. It will be noted that the effects of the irrigation treatments apparently differed widely in the two seasons. In 1929 the difference in the percentages of flowers with 4 carpels, due to the method of irrigation, was much greater in the 18-inch-spaced than in

the unthinned plants; whereas in 1928 there was practically no difference due to irrigation treatment in the 18-inch-spaced plants, and the unthinned plants showed a difference of about 15 percent. This apparent reversal can not be explained, but it was probably due in part to differences in seasonal conditions. Moreover, the data for the two seasons were secured by different methods, one table being based on 300 bolls without regard to the date of their appearance and the other on a considerably larger number of flowers, which appeared during the growing season.

SUMMARY

Practically all the bolls produced by American upland varieties of cotton have either 4 or 5 locks or carpels. The number of locks, although regarded as a heritable character, is affected by cultural conditions. Several investigators have noted the effects of changed or unfavorable conditions of growth upon various characters of the cotton plant. Among the effects observed was reduction in the number of locks per boll.

An example of the effect of close spacing or crowding of cotton plants upon the number of locks was observed by the writers in 1926, in experiments at the United States Acclimatization Field Station at State College, N.Mex. In two of these experiments it was found that the closer spacings consistently produced higher proportions of 4-lock bolls, and these relations were studied more intensively in the following year. Comparisons of lock numbers were made in four spacings: Single plants 12 inches apart in the row; two plants to 12 inches; plants left in groups, or "blocked-out"; and plants left without thinning. No difference was shown between one and two plants at 12 inches, but the experiment with blocked-out plants showed 10 percent more 4-lock bolls than the 12-inch spacing, while the plants that were not thinned showed nearly 17 percent more 4-lock bolls. Numerous blocks were compared and as a general rule the percentage of 4-lock bolls was higher or lower as the number of plants per foot in the rows increased or diminished. Further data along the same lines were obtained in 1928.

In addition to observations in the regular spacing tests in 1928, another experiment was conducted for studying the combined effects of different irrigation and spacing treatments in relation to the production of 4-lock bolls. Two widely different spacings of the plants and two methods of irrigation were employed in this experiment, the spacings being (1) one plant to 18 inches and (2) unthinned plants, and the irrigation methods being (1) normal and (2) "light." In the light-irrigation method, the moisture supplied to the plants was less than is normally required. It was again found that the closer spaced plants had produced considerably higher percentages of 4-lock bolls, but it was also evident that the irrigation practice had had an effect, the light-irrigated plants producing more 4-lock bolls than those which received a normal amount of water. The effect of spacing, however, was evidently greater than that of irrigation.

In 1929 the effect of spacing in the regular tests was again studied, and similar data were obtained as in the two preceding years. In addition, the combined spacing and irrigation experiment was repeated, and the difference in irrigation treatments was intensified by applying still less water to some of the plants. Since it is possible

that on account of differential shedding of 4-lock and 5-lock bolls the effects of cultural conditions may be shown more definitely in the flowers than in the bolls, and in order to obtain a larger array of data, a different procedure was followed. Instead of waiting until the end of the season a daily examination was made of the flowers as they bloomed, the number of carpels being determined by the stigma lobes. On the basis of daily flower counts a large number of comparisons between spacing treatments was made and in much the greater number of cases the close-spaced plants produced more flowers with 4 carpels. Similar daily comparisons of the effects of irrigation treatment showed higher percentages of 4-carpel flowers among the plants under restricted moisture conditions, although in not so many instances as under the different spacings. Average percentages of flowers with four carpels covering the entire season as affected by spacing showed differences of nearly 25 percent in the stressed sections and a little more than 30 percent in the normally irrigated sections, while under the different irrigation treatments the differences were about 3½ percent in the unthinned plants and a little over 9 percent in the 18-inch spaced plants.

The data, on the whole, indicate that the amount of space available for the development of the plants has a material influence upon the proportion of 4-lock bolls produced; crowding the plants in the rows reduces the percentage of 5-lock bolls. The amount of moisture in the soil is also a determining factor, although apparently it is of less importance than the spacing of the plants.

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LYSIMETER EXPERIMENTS WITH SULPHATE OF AMMONIA AND NITRATE OF SODA[1]

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INTRODUCTION

An advantage sometimes attributed to sulphate of ammonia as a nitrogenous fertilizer when compared with nitrate of soda is that it does not occasion so large a loss of nitrogen in drainage water as does the nitrate. It was decided to submit this to trial in the lysimeter tanks at this experiment station. As the drainage from these tanks consists of the percolation of natural rainfall, a test covering a period of 10 or more years should demonstrate what could be expected from a given soil under a rainfall of 30 to 35 inches.

PREVIOUS INVESTIGATIONS

There have been a number of investigations of this subject, but few of them have extended over any considerable period of years. Some of the experiments have been conducted with soils on which plants were grown; others have been carried on with moist and bare soils which were allowed to stand for longer or shorter periods in a percolation apparatus of some kind and then leached. The literature bearing on this subject was reviewed by Bizzell [2] in 1926. Since then some further work has been reported. In general the results indicate that more nitrogen is found in the drainage water from soil treated with nitrate of soda than from similar soil which received an equivalent quantity of nitrogen in the form of sulphate of ammonia. At least such findings are reported by Collison and Walker (*4*), Hall (*7, p. 235–236*), Morgan, Street, and Jacobson (*9*), Collison (*3, p. 815*), Geilmann (*6*), Eckart (*5*), and Tidmore and Williamson (*12, p. 38–41*). The opposite result was obtained by Peck (*10*) and by Geilmann (*5*) when working with clay soils. Geilmann attributes this result to the deflocculating action of the large quantity of nitrate of soda used in the experiment which prevented the soil so treated from draining properly, and in consequence the volume of drainage was small as compared with that of the soil treated with sulphate of ammonia.

Experiments dealing with the effect of these two fertilizers on the removal of calcium in the drainage water have been reported by Hall and Miller (*8*), Morse (*10*), Morgan, Street, and Jacobson (*9*), Eckart (*5*), and Peck (*11*). All of these investigators found more calcium in the drainage water from the soil to which sulphate of ammonia was applied than from soil treated with nitrate of soda, when both were used in amounts containing equivalent quantities of nitrogen.

[1] Received for publication Jan. 26, 1933; issued August 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 63.

EXPERIMENTAL PROCEDURE

Six tanks, each having a capacity of 3½ tons of soil, a depth of 4 feet, and an area somewhat more than 4 by 4 feet, were used for the experiment. The tanks were filled with Dunkirk sandy gravelly loam, the surface soil and subsoil being placed in the order of their occurrence in the field. As its name connotes this soil is a sandy one, only weakly buffered, well aerated, and readily permeable to water. It is a type of soil that is frequently used for market gardening as it responds readily to commercial fertilizers. The tanks were filled with soil in 1921, and the following year the cropping system was begun.

At the beginning of the experiment three nitrogen carriers were used. These included dried blood in addition to sulphate of ammonia and nitrate of soda. After 1925 the use of dried blood was discontinued. Of the two tanks to which it had been applied (nos. 1 and 22), one afterwards received sulphate of ammonia and the other nitrate of soda. It was thought that by giving each a different treatment they could be continued in the experiment without favoring one or the other of the two nitrogen carriers remaining in the test. Moreover, by retaining these tanks in the experiment each treatment could be made in triplicate.

In order to insure a removal of nitrogen in measurable quantities, the applications of nitrogenous fertilizers were large, and to avoid injury to the growing plants the applications were made twice during the season. For this reason it was desirable to have two crops each year, and this could best be accomplished by using market-garden crops. One application was accordingly made in the spring and the other before the second crop was planted in July.

At the outset of the experiment the fertilizers applied each year contained 82.35 pounds of nitrogen per acre. Later the nitrogen was increased to 164.7 pounds per acre each year. This increase in nitrogen was found to be necessary in order to insure an excess over the quantities used by the crops. Somewhat later the quantity of muriate of potash was also increased to insure an excess of potassium, and the quantity of superphosphate was slightly reduced. Whereas the muriate of potash was applied at the rate of 100 pounds per acre at the beginning it was increased to 200 pounds, and the superphosphate, which was used at the rate of 500 pounds, was later decreased to 400 pounds for each crop.

The soil in the tanks was an acid one, and it was considered best to lime it to make it more favorable for nitrification. Accordingly, ground limestone was applied to all tanks at the rate of 4,000 pounds to the acre before the first crop was planted in 1922. The limestone contained 95 percent calcium carbonate and 2 percent magnesium carbonate and was so ground that 94 percent would pass through a $\frac{1}{50}$-inch opening. In the spring of 1926 all the tanks were again limed, this time at the rate of 2,000 pounds to the acre. From that time on only the tanks which received sulphate of ammonia were limed. It was not until 1927 that the tanks treated with ammonium sulphate began to increase in acidity. It then became difficult to prevent the soil in these tanks from becoming strongly acid. In the spring of 1928 another application 2,000 pounds per acre of ground limestone was given to tanks 1, 21, and 24, and in the fall 4,000 pounds

per acre was applied. Again in 1930 an application of 4,000 pounds per acre of ground limestone was made. The total quantity of ground limestone used on the soil that received nitrate of soda was therefore at the rate of 3 tons per acre and on the ammonium sulphate-treated soil 8 tons.

DETERMINATION OF THE SOIL REACTION

Determinations of the hydrogen-ion concentration were made from time to time, this being done more frequently for the sulphate of ammonia soil. The results are shown in table 1.

TABLE 1.—*Hydrogen-ion concentration in soil on different dates, in tanks receiving sulphate of ammonia or nitrate of soda* [1]

Tank no.	Nitrogen carrier	Apr. 21, 1926	Apr. 1, 1927	Nov. 23, 1927	July 12, 1928	Oct. 31, 1928	Apr. 16, 1930	Nov. 25, 1930	May 2, 1931	Nov. 2, 1931
		pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
1	Sulphate of ammonia	6.4	7.4	6.6	6.5	6.1	7.3	7.2	7.0	7.0
21	do	6.3	6.6	6.0	6.1	5.8	7.2	7.2	6.9	6.9
24	do	6.3	7.3	5.7	6.2	5.8	6.6	6.9	6.8	6.8
2	Nitrate of soda	6.5	7.9	7.8			8.1		7.7	7.5
22	do	6.4	7.8	7.7			7.8		7.6	7.6
23	do	6.5	7.8	7.7			7.9		7.7	7.6

[1] In the spring of 1928 ground limestone was applied to tanks 1, 21, and 24 at the rate of 2,000 pounds per acre, and the following fall an application was made at the rate of 4,000 pounds per acre. In 1930 another application was made at the rate of 4,000 pounds per acre.

The determinations of April 21, 1926, were made by the colorimetric method, all the others were made with a potentiometer. Samples were taken from the surface 6 inches of soil. From table 1 it will be seen that there was a strong tendency for the soil that received sulphate of ammonia to become acid. Most of the time the hydrogen-ion concentration in this soil was maintained at a pH of 6.0 or above, but it was only by applying enormous quantities of ground limestone that it could be kept near neutrality. As will be shown later, the quantity of nitrogen applied was not greatly in excess of that removed by the crops and drainage. It was, therefore, not much more than good management would require.

THE CROPPING SYSTEM

Four kinds of plants were used for cropping the soil. Two of these were grown one year and the other two the next year. Thus in 1922 spinach was planted in the spring and carrots in the summer. After the carrots were harvested rye was planted and remained on the land until turned under the following spring in preparation for the next planting. In 1923 lettuce was planted in the spring and this was followed by garden beets. Rye followed the beets and was turned under in the spring. This system was continued until May 1, 1931, at which time the drainage water was collected for the last period recorded in this report.

The crops grew normally. In time of drought enough rain water from the cistern was applied to prevent loss of plants and to maintain growth. All tanks received the same amount of rain water. When seed for the second crop was planted, the ground was usually shaded with muslin to diminish evaporation and protect the young plants. In this way a good stand was obtained.

The drainage water for any year consisted of the quantity collected between May 1 of one year and May 1 of the next. This has been done in all the lysimeter experiments at this station because the drainage water collected during the early spring is greatly affected by the soil treatment of the previous summer. There is little percolation in this climate during the fall and winter months but much when the ground thaws in March and April.

Two constituents only were determined in the drainage water. These were nitrates and calcium. For the former the phenoldisulphonic acid method was used, and for the latter the official method of the Association of Official Agricultural Chemists (*1*). Practically all of the nitrogen in the drainage water was in the form of nitrates.

Drainage water was measured in the receiving cans, each of which had a side tube calibrated to show the contents in liters. An aliquot sample was removed whenever there was enough water to warrant it, and the sample was placed in an acid bottle kept slightly acid with H_2SO_4 to prevent settling out of calcium carbonate.

In the crops dry matter, total nitrogen, and calcium were determined. The official methods of the Association of Official Agricultural Chemists (*1*) were used for these determinations.

EXPERIMENTAL RESULTS

DRY MATTER AND NITROGEN IN THE CROPS

The effect of the nitrogenous fertilizers on the yield of dry matter and nitrogen in the 20 crops is shown in table 2.

TABLE 2.—*Dry matter and nitrogen in the 20 crops grown in soil treated with sulphate of ammonia and nitrate of soda, 1922 to 1931*

Tank no.	Nitrogen carrier	Dry matter	Nitrogen	
		Pounds per acre	*Pounds per acre*	*Percent*
1	Sulphate of ammonia	44,359	937	2.11
21	do	45,232	962	2.13
24	do	45,352	981	2.16
2	Nitrate of soda	53,507	1,069	2.00
22	do	52,513	1,048	1.99
23	do	55,728	1,146	2.06

It is quite evident from table 2 that the crop yields were materially larger in the tanks treated with nitrate of soda and that the quantity of nitrogen removed from the soil was almost correspondingly large. The percentage of nitrogen in the dry matter was slightly higher in the crops grown with sulphate of ammonia. In view of the relative quantities of nitrogen removed in drainage water from the tanks treated with the two nitrogen carriers, which will be shown later, this difference in the amount of nitrogen removed in the crops is interesting.

RELATION OF CROP YIELDS TO HYDROGEN-ION CONCENTRATION IN THE SOIL

While the acidity was greater in the soil treated with sulphate of ammonia than in the soil treated with nitrate of soda during a part of the time consumed by the experiment, such was not always the

case. During the first 4 years there was practically no difference in the soil reaction, as is indicated by the pH determinations made on April 21, 1926. The soil in all tanks had the same reaction at the outset and must have maintained that relation up to the spring of 1926. From 1922 to 1925, inclusive, tanks 21 and 24 received sulphate of ammonia and tanks 2 and 23 received nitrate of soda. The yields of dry matter in the eight crops grown in these tanks during this period are given in table 3.

TABLE 3.—*Average annual yield of dry matter from crops in tanks receiving sulphate of ammonia or nitrate of soda, 1922–25 and 1930–31*

[Data expressed as pounds per acre]

1922–25

Tank no.	Yield from tanks receiving sulphate of ammonia	Tank no.	Yield from tanks receiving nitrate of soda
21	4,325	2	4,884
24	4,203	23	5,118
Average	4,264		5,001
Ratio	100		117

1930–31

21 [a]	5,240	[b] 2	6,107
24 [a]	5,173	[b] 23	6,242
Average	5,206		6,174
Ratio	100		119

[a] pH from 6.8 to 7.3.
[b] pH from 7.6 to 8.1.

The data in table 3 show that the larger yields were in the tanks which received nitrate of soda. The larger yields in tanks 2 and 23 could not be said to be due to differences in the hydrogen-ion concentration of the soil.

Several investigators have found that a marked acidity in soil curtails the effectiveness of sulphate of ammonia, and it was therefore thought that a degree of acidity indicated by a pH value of 6.3 to 6.5 might be sufficient to produce an injurious effect on the growth of the plants in tanks 21 and 24. Accordingly, in 1930 and 1931 the reaction of the soil in these tanks was brought to a point represented by a pH value of 6.8 to 7.3, while that in tanks 2 and 23 was 7.6 to 8.1. As the soil in tanks 2 and 24 had now approached neutrality the reaction should not interfere with the availability of the nitrogen in sulphate of ammonia. The yields of dry matter in the crops grown in tanks 21, 24, 2, and 23 are given in table 3.

The yields of dry matter recorded in table 3 do not indicate that the sulphate of ammonia as compared with nitrate of soda was any more effective when used on a soil with a pH value of 6.8 to 7.3 than when used on a soil with a pH value of 6.3 to 6.5.

NITROGEN IN THE DRAINAGE WATER

All the drainage water that percolated through the soil of these tanks during 9 years was collected, measured, and analyzed for nitrogen and calcium. Nitrogen appeared almost entirely in the form of nitrates. The total quantity of nitrogen from each tank is shown in table 4.

TABLE 4.—*Nitrogen in drainage water from tanks receiving sulphate of ammonia or nitrate of soda, 1922–31*

[Data expressed as pounds per acre]

Tank no.	Nitrogen carrier	Total nitrates	Nitrogen	
			Total	Average annual
1	Sulphate of ammonia	1,236.00	279.34	31.04
21	do	1,204.82	272.29	30.25
24	do	1,034.58	233.82	25.98
Average				29.09
2	Nitrate of soda	1,600.71	361.76	40.19
22	do	1,620.01	366.12	40.68
23	do	1,419.31	320.76	35.64
Average				38.84

The large quantity of nitrogenous fertilizers applied and the sandy nature of the soil combined to cause a large removal of nitrogen in the drainage water. The figures obtained were several times greater than those for nitrogen previously found by the authors in the drainage from a silty clay-loam soil that had received no fertilizer other than a moderate quantity of farm manure.

It is apparent from table 4 that the loss of nitrogen in the drainage was more than 30 percent greater from the tanks treated with nitrate of soda than from those treated with sulphate of ammonia.

It will be recalled that less nitrogen was removed by crops from the soil treated with sulphate of ammonia than from that treated with nitrate of soda. It appears, therefore, that both crops and drainage water from soil receiving nitrate of soda carry off more nitrogen than they do from soil receiving an equivalent quantity of nitrogen in the form of sulphate of ammonia. This is brought out in table 5.

TABLE 5.—*Average quantity of nitrogen removed annually in crops and drainage water from tanks receiving sulphate of ammonia or nitrate of soda, 1922–31*

[Data expressed as pounds per acre]

Tank nos.	Nitrogen carrier	Nitrogen applied—			Nitrogen removed—		
		In fertilizer	In rainfall	Total	In crops	In drainage	Total
1, 21, 24	Sulphate of ammonia	130	6.5	136.5	96.0	29.1	125.1
2, 22, 23	Nitrate of soda	130	6.5	136.5	108.7	38.8	147.5

Table 5 shows that the nitrogen removed in crops and drainage combined was considerably less for the soil treated with sulphate of ammonia than for that treated with nitrate of soda. The quantity of nitrogen applied in fertilizers was somewhat more than the total amount removed from the sulphate of ammonia tanks and less than that removed from the nitrate of soda tanks. In addition to the nitrogen supplied in the fertilizers, the amount brought down by the atmospheric precipitation amounted to 6.5 pounds to the acre.

NITROGEN IN CROPS AND NITRATES IN DRAINAGE WATER IN SUCCESSIVE YEARS

As was to be expected, there were large seasonal variations in crop yields and also in seasonal removal of nitrates from the soil. That there was a fairly uniform relation between these two phenomena is brought out by table 6. Data from tanks 21, 24, 2, and 23 are given because these tanks were treated with the same nitrogen carrier throughout the experiment.

TABLE 6.—*Nitrogen in crops and nitrates in drainage water, during successive years from tanks receiving sulphate of ammonia or nitrate of soda*

[Data expressed as pounds per acre]

Year	Nitrogen in crops		Nitrates in drainage	
	Tanks receiving sulphate of ammonia (nos. 21, 24)	Tanks receiving nitrate of soda (nos. 2, 23)	Tanks receiving sulphate of ammonia (nos. 21, 24)	Tanks receiving nitrate of soda (nos. 2, 23)
1922	52	51	173	220
1923	107	110	33	34
1924	100	118	38	50
1925	90	111	37	43
1926	67	81	300	367
1927	122	159	104	76
1928	76	88	221	319
1929	149	142	44	54
1930	71	92	169	348

The most evident relationship to be seen is that in the years when the crops contained a large number of pounds of nitrogen the quantities in the drainage water were small, and, conversely, when the crops contained little nitrogen the drainage water contained a great deal. This is what might be expected when weather conditions curtail plant growth. Under such circumstances the nitrate nitrogen not absorbed by the plants is carried off in the drainage water.

In every year except one there was more nitrate in the drainage from the nitrate of soda tanks than from those that received sulphate of ammonia. In years when there were small amounts of nitrogen in the crops the removal of nitrogen in the drainage water was such as to entail a considerable monetary loss. This was especially true when the soil was treated with nitrate of soda.

The largest removal of nitrogen in drainage in any one year amounted to 68 pounds to the acre from the sulphate of ammonia tanks and 83 pounds to the acre from the nitrate of soda tanks.

CALCIUM IN CROPS AND IN DRAINAGE WATER

Determinations of calcium were made in the crops and in the drainage water in order to ascertain the relative effects of the two carriers of nitrogen on the removal of this constituent from the soil. Table 7 shows the average amount of calcium removed annually from each tank during the period 1922 to 1931, inclusive. The records for 20 crops and the drainage records for 9 years are included in the table.

TABLE 7.—*Average quantity of calcium removed annually in crops and drainage water from tanks receiving sulphate of ammonia or nitrate of soda, 1922–31*

[Data expressed as pounds per acre]

Tank no.	Nitrogen carrier	Calcium removed	
		In crops	In drainage water
1	Sulphate of ammonia	40.8	357.7
21	do	41.7	385.3
24	do	44.6	359.0
	Average	42.4	367.3
2	Nitrate of soda	31.5	279.2
22	do	34.5	290.7
23	do	34.5	265.8
	Average	33.5	278.6

It is apparent that the crops produced on the soil which was treated with sulphate of ammonia contained more calcium than did those on the soil treated with nitrate of soda, and this despite the fact that larger crops were produced on the latter soil. The drainage water from the ammonium sulphate soil also contained more calcium than that from the nitrate of soda soil. It seems rather curious that with such excessive quantities of soluble calcium in these tanks, as is indicated by the figures for calcium in drainage water, there should have been any greater absorption of calcium by the plants in the sulphate tanks than in the others. It will be remembered, however, that more lime was applied to the sulphate of ammonia tanks than to those that received nitrate of soda. This may account for the larger quantity of calcium in the plants and in the drainage water of the tanks that received sulphate of ammonia. However, from 1922 to 1927, inclusive, both tanks had received the same quantity of lime. A comparison of the calcium removals in the crops and drainage water during these years should therefore indicate what effect, if any, sulphate of ammonia exerted on the removal of calcium. These data are shown in table 8, the figures being given for each year separately.

The same tendency noted in table 7 is apparent in table 8.

In every year except the first the removal of calcium in the crops and drainage water was greater from the tanks that received sulphate of ammonia than from those that received nitrate of soda. Moreover, from 1922 to 1925, inclusive, there was little or no change in soil reaction. The evidence of these years indicates that the addition of sulphate of ammonia is in itself enough to cause a greater removal of calcium, although in later years the application of lime may have contributed to the same end.

TABLE 8.—*Calcium removed in crops and in drainage water, during successive years, from tanks receiving sulphate of ammonia or nitrate of soda*

[Data expressed as pounds per acre]

Year	Calcium removed in—					
	Crops from tanks receiving sulphate of ammonia (nos. 21, 24)	Crops from tanks receiving nitrate of soda (nos. 2, 23)	Drainage from tanks receiving sulphate of ammonia (nos. 21, 24)	Drainage from tanks receiving nitrate of soda (nos. 2, 23)	Crops and drainage from tanks receiving sulphate of ammonia (nos. 21, (24)	Crops and drainage from tanks receiving nitrate of soda (nos. 1, 23)
1922	11.9	11.6	294.5	316.2	306.4	327.8
1923	29.9	23.7	208.5	188.7	238.4	212.4
1924	50.8	61.2	383.9	302.2	434.7	363.4
1925	36.0	30.1	312.7	235.0	348.7	265.1
1926	25.7	24.8	631.4	447.4	657.1	472.2
1927	50.8	36.1	521.7	289.4	572.5	325.5

It would naturally be assumed that the concentration of calcium in the drainage water was greater in the flow from the sulphate of ammonia tanks than from the others. Calculations, when weighted for the drainage measurements and for the calcium found in all of the tanks for the period 1922–31 give the calcium concentration for the water from the sulphate of ammonia tanks at 98.5 parts per million and from the nitrate of soda tanks at 72.5 parts per million.

SUMMARY

Six lysimeter tanks each holding 3½ tons of soil were used in an experiment to ascertain whether as much nitrogen was removed in drainage water from a sandy soil fertilized with large quantities of sulphate of ammonia as from the same soil fertilized with an equivalent quantity of nitrate of soda. The experiment was begun in 1922 and concluded in 1931.

At the beginning of the experiment, the annual application of nitrogen amounted to 82.35 pounds per acre. Later, nitrogen was increased to twice that amount. Ground limestone was applied to all tanks at the rate of 2 tons to the acre at the beginning of the experiment and 1 ton in 1926. After 1926 no lime was given the soil receiving nitrate of soda, but the soil receiving sulphate of ammonia was eventually given 8 tons in an effort to prevent it from becoming too acid.

The hydrogen-ion concentration of the soil did not change materially from the beginning of the experiment in 1922 up to 1926. From that time on there was a constant tendency for the soil treated with sulphate of ammonia to become more acid, and to counteract this tendency 5 tons more of limestone were applied to the soil so treated.

The cropping system consisted of spinach planted in the spring, followed by carrots in the summer. After the carrots were harvested, rye was sown, allowed to grow until spring, and then turned under. This cropping was alternated with lettuce planted in the spring, followed by garden beets in the summer, and rye again grown as a green-manure crop.

Determinations of nitrogen and calcium were made in the drainage water; in the crops dry matter, nitrogen, and calcium were determined.

The average quantity of nitrogen removed annually in drainage water during the entire experimental period was approximately 29 pounds to the acre from the soil that received sulphate of ammonia and nearly 39 pounds from the soil that received nitrate of soda.

In the tanks treated with nitrate of soda crop growth was appreciably greater than in those treated with sulphate of ammonia. The crops in the nitrate of soda tanks removed about 108 pounds of nitrogen to the acre annually, and the crops in the sulphate of ammonia tanks removed 96 pounds. The total average annual removal of nitrogen in crop and drainage combined amounted to between 147 and 148 pounds per acre from the nitrate of soda tanks and 125 pounds from the sulphate of ammonia tanks.

From 1922 to 1925, inclusive, the hydrogen-ion concentration of the soil treated with sulphate of ammonia was approximately the same as that of the soil to which nitrate of soda was applied (pH 6.3 to 6.5). During this period the crop yields in the sulphate of ammonia tanks were smaller than in the nitrate of soda tanks, the ratio being 100 to 117. During 1930–31 the pH readings of the soil in these sulphate of ammonia tanks were 6.6 to 7.3 and those in the soil of the nitrate of soda tanks were 7.5 to 8.1. In these years the crops in the soil receiving sulphate of ammonia still yielded less than those in the soil receiving nitrate of soda, the ratio in this case being 100 to 119. There did not appear to be a relatively better growth produced by the sulphate of ammonia when the soil reaction approached neutrality than when it was more decidedly acid.

In the years in which the crop contained a large amount of nitrogen the quantity in the drainage water was small, and conversely, when the crops contained little nitrogen the quantity in the drainage water was large. The largest removal of nitrogen in drainage in any one year amounted to 68 pounds to the acre from the tanks that received sulphate of ammonia and 83 pounds from those that received nitrate of soda.

Although larger crop yields were produced in the soil that received nitrate of soda, these crops contained less calcium both in percentage and by weight than the crops from soil that received sulphate of ammonia. Moreover, the drainage water from the soil receiving ammonium sulphate contained more calcium than that from soil receiving nitrate of soda. This relationship was recorded both for the period 1922–27, when all the tanks received the same quantities of limestone, and for the period 1922–25, when there was little or no difference in soil reaction in any of the tanks. It may therefore be considered to be associated with the effect of the sulphate of ammonia. The average concentration of calcium in the drainage water from the sulphate of ammonia tanks was 98.5 parts per million, and that from the nitrate of soda tanks was 72.5 parts per million.

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